ABC

Proceedings of the ATMOSPHERIC BIOLOGY CONFERENCE

April 13–15, 1964

UNIVERSITY OF MINNESOTA

Minneapolis, Minnesota

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H. M. TSUCHIYA, Chairman
ALLAN H. BROWN, Co-Chairman

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ATMOSPHERIC BIOLOGY CONFERENCE

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Contents

Preface

The Atmospheric Biology Conference was held April 13 to 15, 1964, at the University of Minnesota under the sponsorship of the National Aeronautics and Space Administration and the University of Minnesota. These proceedings include the papers read and the discussions that followed the presentations.

One of the reasons for NASA's interest in atmospheric biology is to learn more about the types and numbers of biological forms that exist in the atmosphere and the mechanisms whereby these biological forms are injected into, distributed by, and maintained in the atmosphere. Similar questions also interest biologists who investigate the soil and fresh and marine waters.

A second reason stems from the need to implement NASA's program in exobiology which is directed toward search for extraterrestrial life. Much has been written concerning the processes of origin and development of life on the earth but has necessarily been of speculative nature. The space age affords an unprecedented opportunity to test some of these speculations. Do other planetary bodies, e.g., Mars, support life and might they reveal exotic biological forms as did, say, Australia when it was discovered and explored? The technology of sending space probes to other planetary bodies appears to be close at hand but such probes will require sterilization and maintenance of freedom from free-living contaminants until these probes reach such bodies. Information on the height of the biosphere is needed so that ejection of encapsulation (around the probes) will be accomplished at heights above which atmospheric contamination is known not to occur.

As will be evident from perusal of these proceedings, atmospheric microbiology requires interdisciplinary work with cooperative contributions from investigators working in various disciplines, much as has been the practice in oceanography. It will also be evident that much developmental work in devising instruments for detection of life and considerable thought on the analysis of data obtained by such instruments will be necessary.

It is a pleasure to acknowledge the contributions of all of the participants, some of whom came from abroad, and also of the convenors of the several sessions including A. Belmont, W. E. Ranz, C. W. Bruch, and B. D. Church, as well as various individuals of the University who cooperated in making possible this conference. Also the attention to detail and overall quality of the book is due to Mrs. L. Sukalo who served as the editor. Acknowledgment is made also for the valuable help of Mrs. W. C. Galbus, who assisted the editor.

Minneapolis, Minn.
January, 1965

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The Atmospheric Environment

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Abstract

Atmospheric properties of temperature, density, and the accompanying field of radiation as related to the biological environment is discussed. Natural injection of biological material at base of atmosphere will be determined by a complex interaction of properties of the biological material itself and particular characteristics of atmosphere at surface of earth. Extreme variability of atmospheric surface layer is emphasized.

While it is predicted, therefore, that for most pollens and spores there would be a rapid decrease in concentration with altitude, particles approaching micron size might be expected in concentration at tropopause almost half as great as near surface.

Some additional environmental hazards encountered by small particles at high altitudes will therefore also be discussed.

Introduction

I shall assume that you are familar with the properties and characteristics of the atmosphere near the surface of the earth and in this paper shall concentrate on the planetary aspects of the atmosphere.

Extent of Atmosphere

The first point I wish to make is that the atmosphere is a relatively thin shell surrounding the earth and that its vertical dimension is small compared to its horizontal or surface dimensions. The radius of the earth is about 6,000 km and, while there is no lid and therefore no precise upper boundary on the atmosphere, for most purposes one might consider the atmosphere to end at an altitude of about 300 km. The atmosphere is held onto the surface of the earth by the gravitational attraction of the earth upon the molecules of the air and although one can't give a precise figure for the depth of the atmosphere, it is possible to state with considerable accuracy that the mass of the atmosphere is very nearly 1 kg/cm^2 over the surface of the earth. Roughly, the mass is equivalent of the mass contained in the first 20 ft or so of the solid surface of the earth. The total mass of the atmosphere is only a small fraction of the mass of the oceans, perhaps only a fraction of a percent of the mass of all the water on the planet. The primary constituents of the atmosphere are of course the classical perfect gases, oxygen and nitrogen.

Atmospheric Density

The density of the atmosphere decreases in the vertical. The atmospheric gases are compressible so that decrease in density is most rapid near the surface of the earth and less rapid at higher altitudes. A graph of density with altitude is only moderately instructive because of the wide range of density to be shown on a single graph. A diagram of density versus altitude is shown in Figure 1.

The graph of density is very nearly an exponential curve, the analytical expression for which is much more than the graph itself. The approximate formula for this curve is simply

$$\rho(h) = \rho_o e^{-h/H}, \qquad \text{I}$$

expressing the fact that the density at some altitude (h) is equal to the surface density (ρ_o) times (exp-h/H).

Gaseous gravitational atmospheres tend to follow such an exponential law. Astronomers generally call the factor H, which represents the rate of decrease of the exponential, the scale height of the atmosphere. This means that at an altitude, h = H, the density will have decreased to approximately a third (1/e) of its value at the surface of the earth. As a good rule of thumb for the first 100 km of the atmosphere, one can use a value of H, the scale height, of 8 km. The pressure also, which is proportional to density, decreases exponentially with

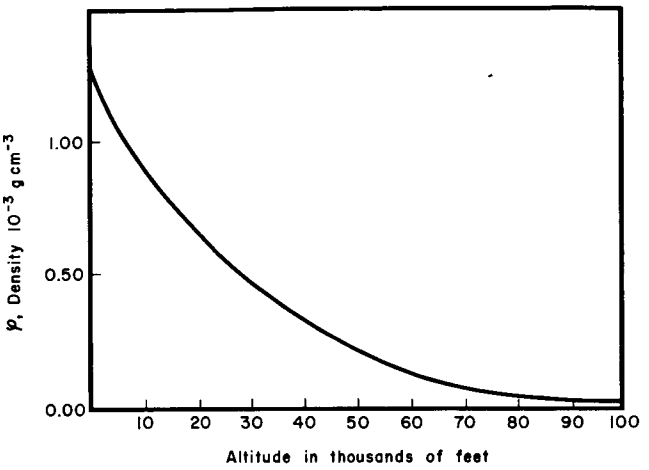


Fig. 1. Variation of density with altitude (Adapted from Handbook of Geophysics, 5).

height and will decrease by a factor of one-third for every 8 km increment of altitude. Modern jet planes normally operate at altitudes of about 8 km or a pressure of about one-third of the surface pressure. Properties of the atmosphere that depend directly upon density or pressure will of course obey the same law. The mean free path, for an example, which is proportional to the reciprocal of the density, will increase exponentially.

The meteorologist arrives at Equation I in the following way. He notes first that the rate of change of pressure with height is simply proportional to the weight of the atmosphere per unit volume, i.e.:

$$\frac{dp}{dh} = -\rho g \qquad \text{II}$$

He further recognizes that the atmosphere behaves approximately as a perfect gas and therefore the decrease of pressure with height is proportional to the pressure itself; he thereby arrives at the barometric formula, which has the same form as Equation I:

$$\rho(h) = \rho_0 e^{-\frac{Mg}{RT}h} \qquad \text{III}$$

Equation III therefore indicates that the astronomer's scale height (H) really depends upon the gas, the absolute temperature, and the acceleration of gravity (g).

$$H = \frac{RT}{Mg} \qquad \text{IV}$$

In the derivation of Equation III, it was assumed that the atmosphere was isothermal with a constant molecular weight and therefore a constant scale height. While the atmosphere may seem anything but isothermal to biologists, on a gross scale the atmosphere below 100 km is isothermal (the absolute temperature does not vary by 25%) and the scale height does not vary from the mean value of 8 km by more than 1 km.

Equation I can be obtained in a different way. By kinetic theory one can predict for thermal equilibrium that the number of molecules should be distributed exponentially according to potential energy as given by the classical expression:

$$N = N_0 e^{\frac{-mgh}{kT}} \qquad \text{V}$$

where m is the mass of the molecule and k is Boltzmann's constant.

Let me emphasize that Equation V is a prediction of kinetic theory and which is, of course, very nearly identical with the barometric formula Equation III obtained by the meteorologist for an isothermal atmosphere. Equations III and IV are exactly the same if the atmospheric gas has a constant molecular weight. The atmosphere, of course, is a mixture of molecules of different molecular weights so that by kinetic theory one would predict a slightly different scale height for the nitrogen in the atmosphere than for the oxygen in the atmosphere. Actually, a separation by molecular weight (usually called gravitational separation) is not observed in the atmosphere, at least below 100 km. The tendency for establishment of thermal equilibrium and corresponding isothermal state with the gravitational separation is largely counteracted by large scale mixing in the atmosphere. Another way of describing this phenomenon is to say that the lowest 100 km of the atmosphere undergoes constant mixing, which is much larger than the rate of molecular diffusion.

Vertical Temperature Distribution

The major characteristics of the vertical temperature distribution are shown in an example of a rocket measurement of temperature made by the Signal Corps in Figure 2 (4). About 100 conventional balloon observations are made twice each day; this example is fairly typical of the altitudes that are attained. The U.S. Weather Bureau strives to achieve an altitude of about 30 km on these daily flights. Experimental balloons can reach altitudes of 45 km, but at a prohibitive balloon weight to payload ratio. To measure above this level, as in this case, it is necessary to use rockets.

To help in the orientation of the density or pressure with this graph (Fig. 2) of altitude and temperature, let me remind you that the density goes down about one order of magnitude for every two scale heights and therefore at an altitude of 16 km just about the first minimum of temperature, the density is down by a factor of 10 and the pressure is therefore about 100 mb as compared to the surface value of 1,000 mb.

Temperature is measured in the conventional balloon soundings by measuring the resistance of a small ceramic cylinder. Conventional temperature sensors can be used to an altitude of about 50 km and then fail because the mean free path generally is larger than any measuring instrument and it is impossible to make thermal contact with the air molecules. The upper points of this sounding were

made by measuring the speed of sound transmitted from explosions set off by the rocket during its ascent.

The characteristics of the vertical temperature distribution may be summarized as follows: 1) There are two minima with temperatures of near 200K; one at about 12 km and one at about 80 km altitude and an intermediate maximum at 50 km where the temperature approaches the same value as at the surface of the earth. 2) The lowest scale height of the atmosphere is characterized by a decrease in temperature with altitude. 3) In the standard atmosphere or the engineer's average atmosphere, the temperature decreases 6.5 C/km. 4) There is a transition from a lapse rate of 6.5 °/km to a near isothermal layer at about 12 km (Fig. 2). The transition from lapse to isothermal conditions characteristically is abrupt as shown on the balloon sounding although the abrupt transition becomes smoothed out in the average (smooth curve of Fig. 2). The abrupt break is called the tropopause and the layer above is called the stratosphere. The region of the temperature maxima near 50 km is generally called the mesosphere although the nomenclature for the layers above the stratosphere does not always have universal acceptance. Above 100 km, the limit of the diagram, decomposition of the atmosphere begins to take place; this region of chemical reaction is frequently termed the chemosphere.

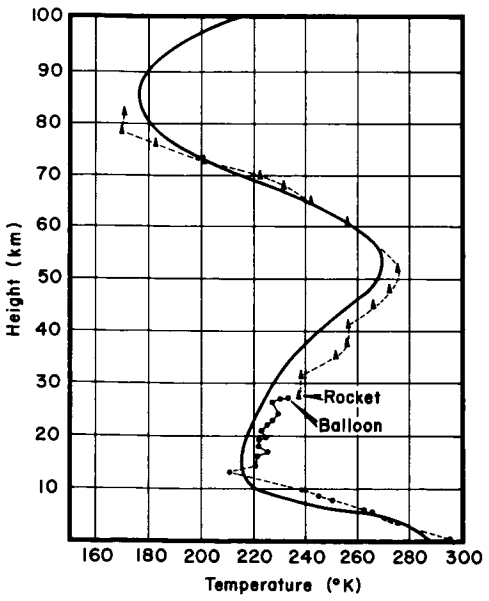
Radiation Field

The field of radiation is not only a physical parameter of interest in itself but it is basic for an understanding of the vertical temperature distribution. The radiation from the sun is very much like the radiation from a black body at a temperature of about 6,000 A. The peak in the solar spectrum (Fig. 3) occurs in the middle of the spectrum of visible light, about 5,000 A. While some radiation is emitted beyond the wavelength extremes shown on this graph (2,000 - 18,000 A), the amount of energy contained in these wavelengths is low.

It is important to remember that the interaction of electromagnetic radiation with matter is not continuous, but occurs in increments and is dependent upon wavelength. In the wavelength region of visible light, (in which most of the solar energy occurs), the components of the atmosphere are not reactive so that solar radiation arrives at the surface of the earth with little attenuation. The earth's surface is relatively absorbent to solar radiation and converts this radiant energy into heat so that our atmosphere is really heated from below. The atmosphere will heat up until it reaches a condition of thermal equilibrium where it transfers heat back to space at the same rate at which it is received.

Since the transfer of heat usually occurs down a temperature gradient, we would expect a decrease in temperature with height just as observed for the troposphere. This explanation is, of course, an oversimplification but one should expect a decrease in temperature with altitude simply from the fact that atmosphere is heated from below. Once the solar radiation is converted into heat, the energy must ultimately be transferred back to space by radiation, but the radiating material is at a much lower temperature than the sun so that the wavelength characteristic of this radiation is quite different than the wavelength characterizing the solar radiation.

Figure 4 is a schematic graph of spectral intensity of the solar radiation and of the earth radiation on a logarithmic wavelength scale. The peak of the solar spectrum radiation occurs at



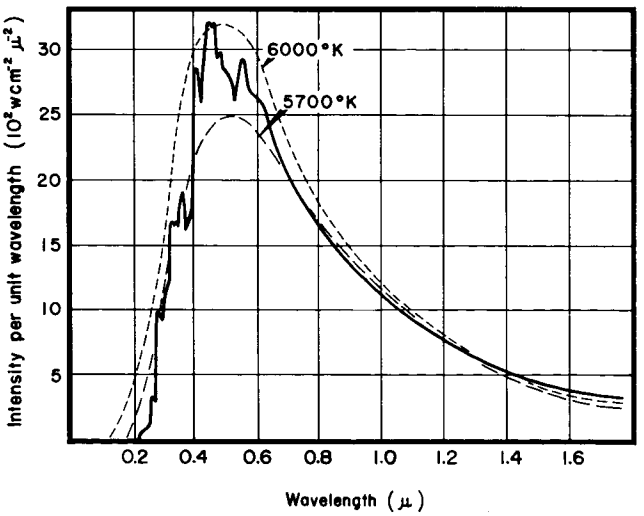


Fig. 2 *(left)*. Example of vertical temperature distribution (adapted from Stroud et al., 4). Points indicate actual observations; balloon observations (*circles*) made by conventional meteorological soundings. *Solid line* is average value for atmosphere.

Fig. 3 *(right)*. Solar spectrum (adapted from Johnson, 3). *Dashed lines* are spectrum of black body at 5,700 and 6,000 K.

0.5 μ , while the peak of the earth and atmosphere radiation is at 10μ corresponding to a black body of about 250K.

The radiation field of the atmosphere thus has two components of about equal flux in distinct and separate wave bands: the solar or visible radiation and the earth or infrared radiation. Interaction of electromagnetic radiation with the atmosphere occurs only at the very short or ultraviolet end of the spectrum and in the infrared portion of the spectrum while in between, as we already noted, the atmosphere is nearly transparent.

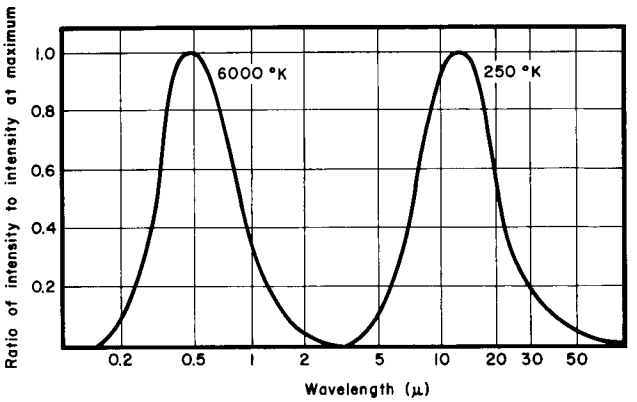


Fig. 4. Schematic spectrums of solar and of atmospheric infrared flux (adapted from Goody and Robinson, 2). Log scale. Solar *(left)* infrared *(right)*.

To complete the discussion of the solar radiation in the ultraviolet, wavelengths shorter than 2,900A are almost completely absorbed in the atmosphere and unobservable at the surface. The solar spectral distribution in Figure 3 is necessarily derived from rocket measurements made above the atmospheric absorption. In the ultraviolet region the solar spectrum contains far less energy than a black body at either 6,000 or even 5,700K (black body curves, Fig. 3) and, while the total energy in the ultraviolet is only about 10% of the solar energy, the precise spectrum is important in the theory of upper atmospheric absorption and ionization. At even shorter wavelengths (< 2,000 A) the solar spectrum is quite variable and at times is vastly enhanced over the 6,000° black body.

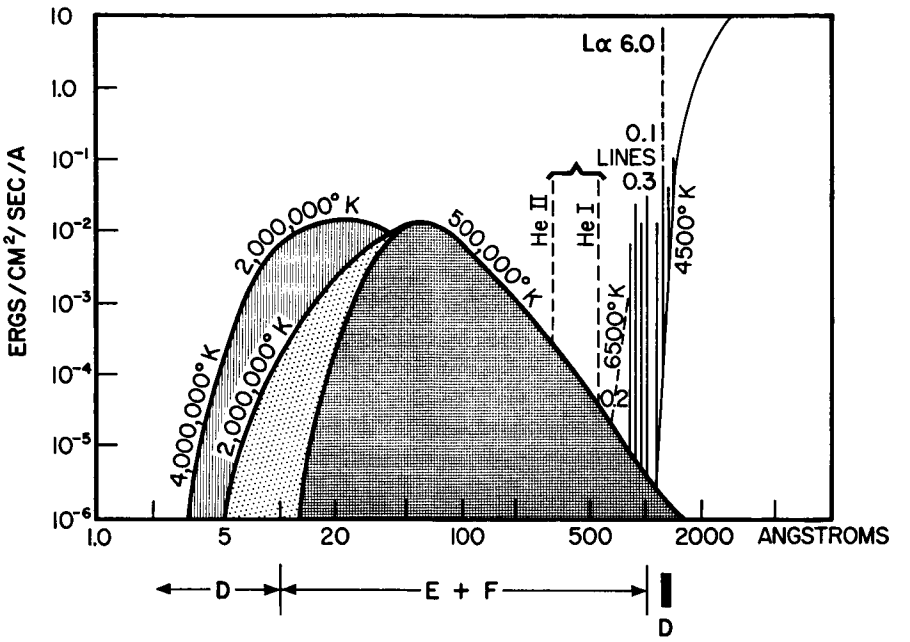
Figure 5 is a diagram of some National Research Laboratory rocket measurements of the solar spectrum during disturbed conditions. Intensity was measured only in fairly narrow bands and then analyzed with the appropriate black body spectrum that would yield this same intensity in the observed band. Typically, temperatures of 1 to 1/2 million degrees would be required to produce the short wavelength spectrum. As a final warning, the diagram in Figure 5 is on a log scale and the total energy observed in these short wavelengths is only a slight fraction of the solar energy.

To illustrate both the degree of atmospheric absorption and the layers in which the absorption occurs, another result of the National Research Laboratory rocket flights is shown in Figure 6, in which is plotted that altitude of the atmosphere at which two-thirds of the incoming solar radiation has already been absorbed. The nature of the absorber has been indicated by the symbols above the corresponding absorption region. The shortest wavelengths are absorbed in the ionization of oxygen and nitrogen mainly above 150 km; the next longer wavelength region is absorbed in the decomposition of molecular oxygen near 100 km, while energy in the 2,000 to 2,900 A region near 40 km is absorbed by ozone (Fig. 6).

It is apparent therefore that exposure to ultraviolet radiation in the atmosphere will not increase substantially until altitudes of above 30 km. On the other hand, most of the energy in the solar ultraviolet is in the ozone absorption region and almost the full solar energy spectrum shown in Figure 3 would be encountered at altitudes above 40 km.

From the previous simplified discussion, we noted that one expects the energy to transfer from higher to lower temperatures. If this statement can be inverted, we deduce that high temperatures in the upper atmosphere must be associated with energy sources other than the surface heating. The temperature maximum at the 50 km mesopeak is primarily due to the absorption of solar energy in the 2,000 to 2,900 A region by atmospheric ozone. Although the total ozone concentration hardly exceeds one part per million, it is very absorbent in this wavelength region. The temperature increase above 100 km is due to the absorption by the breakdown of the oxygen molecule and the ionization of oxygen and nitrogen.

The upward beam of infrared radiation, although containing the same flux as the average incoming solar beam, presumably is of interest here only as it might determine the heat balance and temperature of biological materials. Since the intensity is near that of a black body at 250K, the effect on absorbing materials is generally to warm objects if the ambient air temperature is below 250K. The interaction of infrared radiation with the more complex molecules of the atmosphere, however, is important in determining the heat balance and the equilibrium surface temperatures of the atmosphere. The principal absorbers in the infrared are water vapor and CO_2, which can be excited to rotation or rotation and vibration by radiation in infrared wavelengths. Perhaps not accidently, the outgoing infrared radiation is centered on a window in the water-vapor carbon-dioxide absorption bands. The 10 μ peak lies just between intense absorption bands in the wings of the spectrum; the surface temperature of the air will be held more nearly constant (near 250K) than it would be with different atmospheric infrared absorption characteristics.



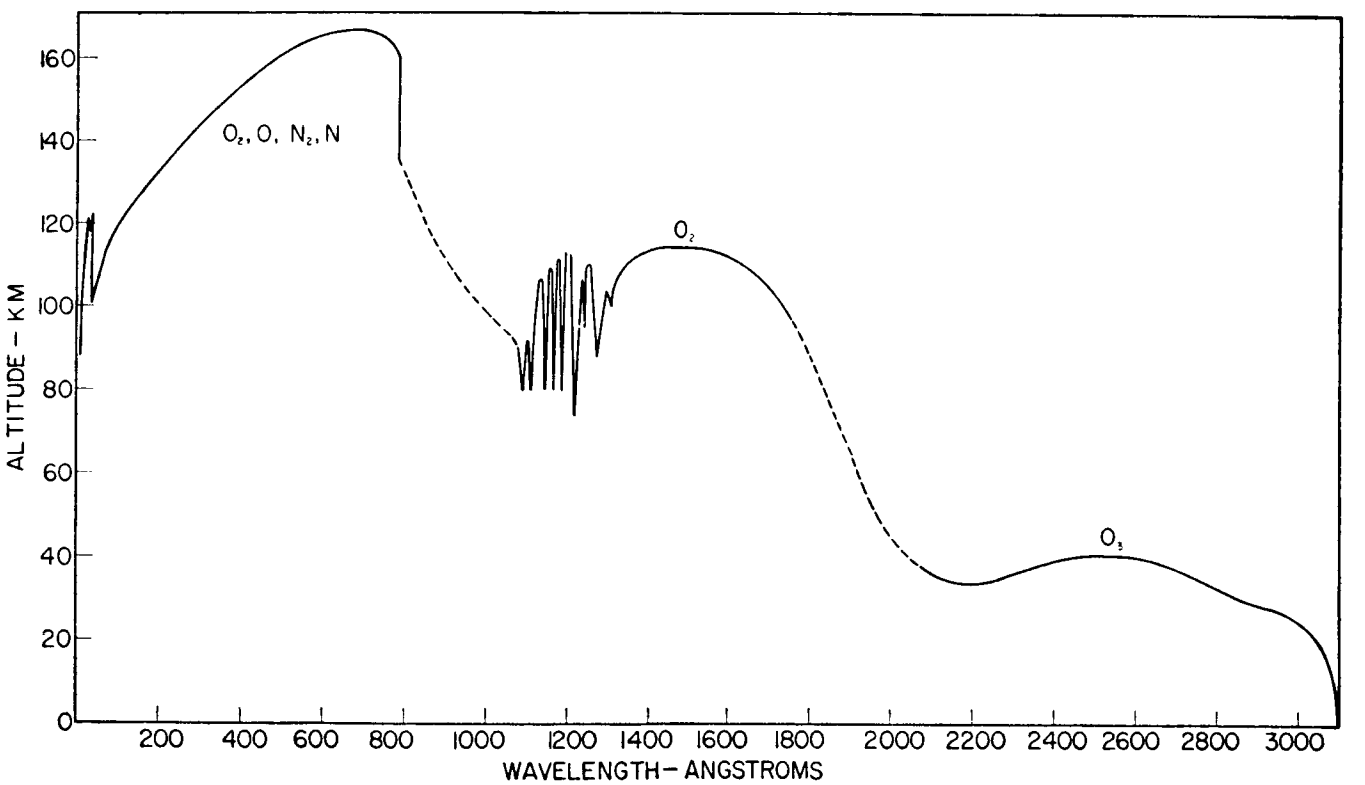


Fig. 5 *(top)*. Solar spectrums at short wavelengths during solar disturbances (Friedman, 1).
Fig. 6 *(bottom)*. Atmospheric absorption in the ultraviolet. Graph shows absorber and altitude at which e^{-1} of incident solar radiation remains (Friedman, 1). Log scale.

Vertical Stratification of Atmosphere

The one further atmospheric characteristic I wish to discuss is its vertical stratification. We have already noted that the nomenclature of the various layers of the atmosphere is based upon the temperature lapse rate. The reason for this classification is not solely convenience but because the temperature lapse rate is so closely connected with the vertical stratification and the associated properties.

Since the atmosphere is a relatively thin layer, horizontal mixing would be expected to predominate over vertical mixing and there is a much greater tendency for properties to become more homogeneous in the horizontal direction as compared to the vertical direction. The marked vertical stratification of the atmosphere, however, can only be accounted for by a discussion of the property termed stability. Generally (except in thunderstorms), the atmosphere will be hydrostatically stable; that is, a parcel of air can be displaced vertically only by the expenditure of work. If somehow a parcel of air is pushed upward, for example, the displaced air will be in an environment of lower pressure and it will expand. Air being a poor conductor of heat, the displaced particle will cool adiabatically.

While Figure 2 is actually a graph of temperature versus height, because pressure is a monotonic function of height, it could equally well be considered a graph of temperature versus pressure or a kind of thermodynamic diagram on which we could plot the path for an adiabatic process. A plot of the isentropic path of the displaced air would show that the temperature decreases at 10 C/km. Recalling that the average tropospheric lapse rate is 6.5 C/km, it should be apparent that a parcel of air displaced upwards will be colder and denser than air in the environment. The displaced air will therefore be subjected to a force directed downwards; i.e., a force directed oppositely to the displacement. The physical definition of stability is the restoring force per unit mass per unit displacement and it is numerically related to the temperature lapse rate.

Thus, the troposphere with a lapse rate of $6.5\,C/km^{-1}$ is much less stable than the stratosphere where the lapse rate is almost zero. Vertical mixing of the stratosphere is therefore much inhibited as compared to the troposphere. If we characterize (as is common in meteorology) the mixing by an effective "eddy" diffusivity, the vertical diffusivity of the troposphere is 10 to perhaps even 1,000 times greater than the stratosphere. (Although the precise value is open to dispute.) The abrupt transition in ozone concentration at the tropopause would tend to support the larger figure.

Once vertical mixing does occur, the mixing itself tends to establish the adiabatic lapse rate of 10C/km so that mixing tends to produce a less stable atmosphere. Mixing, therefore, makes it easier to have further mixing. This nonlinear behavior of the atmosphere for convection is one of the great difficulties that hampers a theoretical treatment of the mixing process. It seems likely that nonlinear behavior would account for the fact that the tropopause generally occurs as a sudden break in lapse rates rather than a gradual transition. The warming of the layers above the tropopause by ozone absorption provides a stable lid to tropospheric convection and there would appear to be a lapse rate beyond which vertical mixing is rapidly damped.

Summary

The properties and characteristics of our atmospheric environment as discussed here are:

I. The atmosphere is a relatively thin shell of gases, primarily oxygen and nitrogen, held on the surface of the earth by attraction of gravity.

II. Air is compressible and the density therefore decreases with height rather rapidly near the surface and then less rapidly in the upper atmosphere. The density distribution with altitude may be characterized for engineering purposes by the exponential law with the scale height of about 8 km.

III. The main features of the vertical distribution of temperatures are two minima, one at 12 km and one at 80 km, with a maximum of about 50 km where the temperature is very similar to that of the surface of the earth. The layers of the atmosphere are classified according to their temperature lapse rate. The first layer above the surface of about 12 km depth is called the troposphere. The break in temperature where the lapse rate becomes more stable is called the tropopause and the region above is called the stratosphere. The transition between the lapse rate of 6.5 C/km in the troposphere, to a value near zero, characteristically occurs as a very sudden break on the individual temperature sounding.

IV. The incoming solar or visible radiation can be characterized as black body radiation at 6,000 K while the infrared or atmospheric radiation can be characterized as black body radiation at 250 K. The interaction of electromagnetic radiation with matter is not continuous and depends upon wavelength. Atmospheric constituents are not reactive at intermediate wavelengths but in the ultraviolet, the molecules decompose and absorb radiation, and at infrared wavelength, some of the lesser atmospheric constituents are very absorbent.

V. The principal features of the vertical temperature distribution are caused by the absorption of solar energy, the surface temperature maximum produced by absorption of the visible radiation by the earth's surface. The intermediate maximum is due to ozone absorption and the high temperatures above 100 km are produced by the decomposition and ionization of oxygen and nitrogen.

VI. The atmospheric properties are much more homogeneous in the horizontal than in the vertical. The vertical stratification is due to the fact that it requires work to displace a particle of air in its environment. The degree of stability is numerically related to the temperature lapse rate. Vertical mixing is relatively inhibited in the stratosphere as compared to the troposphere.

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Nomenclature

h	Altitude
H	Scale height or rate of decrease of exponential (astronomy)
ρ	Density
m	Mass of molecule
M	Molecular weight of gas
R	Universal gas constant
N	Number density of molecules

Discussion

Brown — You describe the atmosphere as if it were all one atmosphere. How much variation is there from season to season and from year to year? Is the variation negligible or is it spectacular?

Mantis — The "standard" atmosphere is just an international "engineers' atmosphere." It is of course not even a true average, but simply an agreed standard of reference. The greatest departures from the standard atmosphere occur in the surface layers so that you presumably are already aware of how far from standard the atmosphere can be. Diurnal variations are largest in the lowest few thousand feet. I have added a diagram (Fig. A) of two soundings made in the morning and in the evening of the same day, at Oklahoma City, to illustrate typical departures from the standard atmosphere. Departures of 10C are common near the surface, and actual soundings invariably possess a large number of irregularities. It is interesting to note that the tropopause is a sharp break on the actual sounding, although it does not occur precisely at the level of the standard atmosphere tropopause.

The greatest horizontal variation from the mean temperature distribution is in the surface layer in a north-south direction, so that the greatest variation is in the lowest layers of the atmosphere, in the troposphere. The stratospheric variation from north to south, however, is almost as great and the gradient is oppositely directed. The temperature at the tropopause is about -55C in mid-latitudes. At the equator tropopause temperature may be as low as -80. Incidentally, -80C is close to the temperature at the upper temperature minimum (at 80 km).

The surprising thing about the mesopeak at 50 km is that even though there is no radiation received to provide this warming in northern regions, in winter the annual variation is less than in the troposphere.

Oswald — Could you elaborate a little more on the ozone layer of the atmosphere where you stated that ozone was absorbing the energy from sunlight? Could you make some statements regarding concentrations?

Mantis — The concentration at the peak is 1 ppm — 10^6. The ozone concentration is down from this figure by roughly a factor of a thousand at the surface. The ozone concentration remains almost constant in the troposphere (that's another indication that the troposphere is fairly well mixed) and then increases quite rapidly right above the tropopause. The ozone concentration looks a lot like the temperature profile. The concentration peaks around 25 km and then it decreases above that. There really is a very small amount of ozone. The ozone, however, about one-fourth centimeter layer at STP, absorbs all the solar energy below 2,900 A. We must use a balloon or another aircraft before we can see the ultraviolet part of the solar spectrum.

Question from audience — This concentration is 1 ppm of what?

Mantis — Of air. One ozone molecule per million oxygen-nitrogen molecules at that maximum peak of 25 km.

Question from audience — How about the oxides of nitrogen? Do any form in the tropopause layer?

Mantis — There are weak nitrous oxide lines in the spectrum; I don't have any figure at all for the concentration. It's very small.

Question from audience — How's the water distribution?

Mantis — The water distribution is a maximum, of course, at the surface. The maximum is something like 2%, the most the warmest atmosphere could hold in vapor phase. The distribution decreases with height. The latest finding seems to be that the relative water vapor concentration begins to increase again above the tropopause (the mixing ratio or the

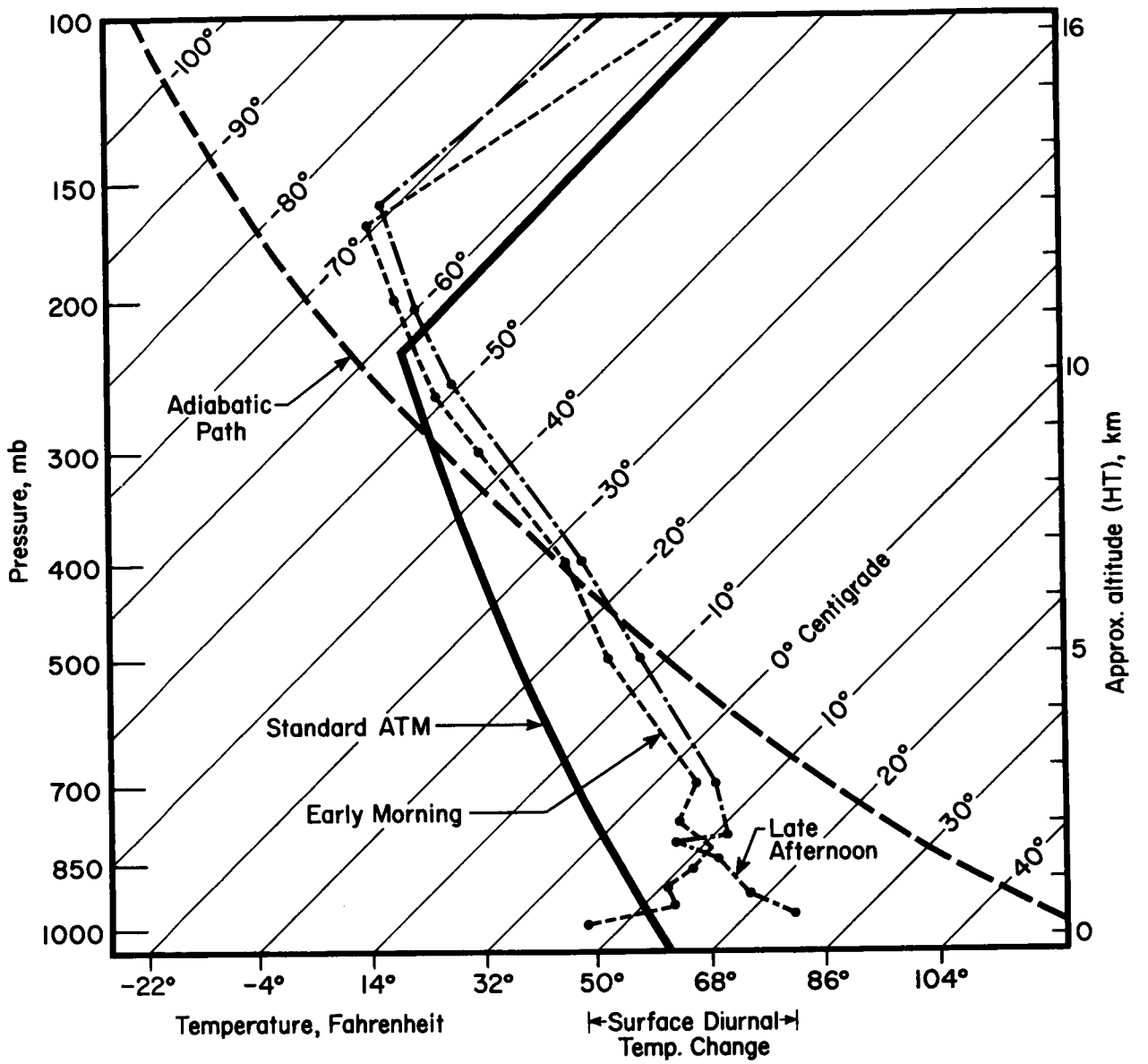

Fig. A. Example of morning and evening vertical temperature distribution at Oklahoma City 7 April to illustrate typical diurnal and seasonal departures from standard atmosphere.

parts per million). I don't know what a good figure is here. It's probably lower than 0.01 parts per thousand.

There is a great decrease and then a slight increase again, which might mean that this is extra-terrestrial hydrogen recombining in the atmosphere.

Question from audience — Is there any variation in the composition with altitude, particularly in regard to carbon dioxide?

Mantis — There is no gravimetric separation in the atmosphere. There is a decrease in water vapor concentration with altitude because the temperature decreases with altitude and the vapor condenses. Some of the measurements of carbon dioxide concentration at high altitudes have been contradictory, but there is fair agreement that the mixing ratio, the ratio of the number of carbon dioxide molecules to nitrogen molecules is constant.

Goetz — Wouldn't this contradict what you described as the kinetic or barometric law?

Mantis — Oh yes. That is right. It was my point that that law is not obeyed. Mixing goes on, up to 80 km. At 80 km one begins to get gravitational separation. One gets quite rapid decomposition of oxygen above this point, so that by 100 km all the oxygen is atomic oxygen rather than molecular.

Goetz – Could you say somewhat more about the ozone distribution, the partial pressure of the ozone below the tropopause, particularly as it occurs close to the earth's surface? Paetzold (B) reported a steep decline of the ozone concentration below the tropopause and barely any at low altitudes. It would be interesting to learn more detail about this profile particularly in the biosphere.

Mantis – There's a good deal of information now about the ozone distribution, but the ozone near the surface is so low (it's right on the verge of measurement limits of the present instrument) that there are few significant fluctuations in their measurements. The ozone distribution will be irregular at the surface, but fairly uniformly low.

I was interested in this myself to see if one could get an average gradient of ozone in the troposphere. Near the surface where one can use more elaborate instrumentation he can get variation in ozone. The ozone is decomposed or is absorbed at the surface, so the ozone that is made in the stratosphere is transported down to the surface, and then decomposed. There's a fairly large diurnal variation of ozone at the surface; at night the lowest few meters of atmosphere form a large temperature inversion which is a stable region. Mixing is therefore reduced and the ozone is just precipitated out; there is no more feeding down from above; the ozone goes down at night. As soon as the sun comes up the ozone comes right up to the ordinary value that is about 1 in 10^9, I guess. One is supposed to be able to smell ozone at 1 in 10^8.

Goetz – I am thinking about the work of F. W. P. Goetz in Switzerland (A), the large fluctuations of ozone concentration he found at his station in Arosa, whenever clouds or fog drifted into the steep alpine valleys.

Mantis – I'm not familiar with that work. Dr. Kroening of the University of Minnesota makes ozone measurements right here at the University. The fluctuations at the surface seem to be almost entirely controlled by vertical mixing.

Junge – Maybe I can give some information on this question. Considering both old and new data it seems as if the mixing ratio of the ozone in the troposphere (on the average) increases approximately by a factor of two when one goes from 1, 2 km to the upper troposphere.

Goetz – I would be interested in what happens in the biosphere, that is, in the last kilometer of the atmosphere.

Junge – In the troposphere the ozone concentrations are more constant than we thought except near the surface. Near the surface there is destruction of the ozone. Depending upon the micrometeorological conditions in the surface layer you might have all values from zero up to the average value.

Mantis – The photochemical detector was used here. The quality of the instrument, the sensitivity, is a function of the amount of money put into the photodetector (one can't fly an expensive one worth $2,000 or so), thus the fluctuations in the troposphere are just on the border of measurement with the regular instrument. I think the fluctuations are real, but the data are touchy.

Question from audience – You said that the ozone is formed near the surface due to photochemical reactions?

Mantis – Also, yes.

Question from audience – There's a small amount of shortwave ultraviolet coming through?

Mantis – Well, there isn't a precise point, but anything less than 2,900 A is really absent at the surface. The ozone is formed in surface air by a complicated photochemical process that involves the hydrocarbons in the air at the surface. It's a sensitized reaction.

Goetz – You need a substantial number of energetic photons?

Mantis – Yes. It isn't the same problem. The formation of ozone in the upper atmosphere is relatively simple. Oxygen decomposes above 100 km and diffuses downward; also some decomposes down to 50 km. Then it recombines with an oxygen molecule to form ozone. There's no problem in understanding the process and this reaction is direct; there must be radiation of very short wavelength to accomplish the decomposition of oxygen. In the case of the reaction at the surface, ultraviolet is not needed.

Soffen – What sorts of devices are used for measuring the temperature above the balloon limits?

Mantis – Up to about 50 km one can use a fine thermistor. The limit of ordinary temperature sensing is 50 km because the instrument is the order of the mean free path; once you pass that limit you can't measure temperature. The sounding I showed was a measurement of the speed of sound. Grenades were set off from the ascending rocket and the temperature sounding reconstructed from the measurement of the time of travel of the explosion.

Soffen – Is that by speed of sound?

Mantis – Yes.

Soffen – You have no density measurement then?

Mantis – No, with the barometric formula the temperature and composition determine the density. On the other hand, at higher altitudes density measurements are made with vacuum gauges and the temperature deduced from the barometric formula.

MacLeod – Is the temperature profile that you showed, the kind of temperature profile that the micron size particles would have at these altitudes?

Mantis – I wanted to make that point, too, that the physics of the heat transfer is such that the smaller the size the more effectively conduction competes with radiation. So that's true. The particles of micron size would be very hard to move away from the air temperature by radiation processes.

Gregory – I think you said that ozone disappeared from the lower part of the atmosphere at night. Could you elaborate on this?

Mantis – The mechanism I propose is the following. As Dr. Junge mentioned, there is a slight gradient of ozone in the troposphere, that is a higher concentration at the tropopause than at the surface. There will be a constant deposition down the gradient onto the surface.

At night a temperature inversion may form in the atmosphere at the surface. This inversion reduces convection and blocks the transfer to the surface layer of air. The ozone in this surface layer then diffuses and deposits on the surface and the concentration decreases. The nighttime decrease in ozone will be restricted to a few meters above the surface. Soundings made by hoisting an instrument up on a balloon show that the usual tropospheric concentrations will still be observed about 100 meters above the surface.

Literature Citations

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B. PAETZOLD, H.-K. & F. PISCALAR. 1958. Berichte des Deutschen Wetterdienstes No. 51: 101 - 104.

Transport and Mixing in Atmosphere

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Abstract

Density of standard meteorological data is described and discussed. It is pointed out that the standard data have limited use for micrometeorological purposes, and that we must employ empirical turbulence and mixing theories to describe natural phenomena. Advantages and disadvantages of classical mixing length theory are discussed, and it is pointed out that it is an assumption that the flux of any quantity is directed opposite to the gradient of the quantity. Examples of momentums and heat transports in the atmosphere giving a transport in the opposite direction are shown.

Standard meteorological data can be used to compute transports of momentum, sensible heat and moisture in the atmosphere. Examples of such calculations are shown. The transports are given as functions of latitude, pressure, and longitudinal wave number. The maximum heat and moisture transports are in lower part of atmosphere, while the maximum momentum transport takes place in upper part of troposphere.

It is furthermore demonstrated that a large fraction of the different transports is carried out by the largest scales of motion (the lowest wave numbers), especially during winter season.

It is the purpose of this lecture to present certain ideas related to transport and mixing processes in the atmosphere. One of the more important concepts to mention immediately is the fact that the transport processes and the mixing which takes place depend very much upon the scale of the atmospheric motions. We should realize from the outset that there is always a lower limit to the scale which we can analyze with a given meteorological network. If we are interested in the micro-processes around a given tree or plant we must naturally measure the quantities in a dense network of observations in order to be able to compute the gradients that are of importance in the transport of any quantity.

The standard meteorological observations consequently have limited use. The density in space varies a great deal from one part of the world to another. In general, we find a greater density of observations over land than over the ocean. The average distance between observations in the Atlantic Ocean is about 900 km while the corresponding distance in the Pacific Ocean is about 1,200 km. In two parts of the world, the eastern United States and Western Europe, we find the best observing networks; even here the average distance between observation stations is about 300 km.

If we therefore want to estimate transport processes as a function of scale from standard observations it becomes important to realize that the lower limit of the scale that we can expect to consider in a realistic way will be about 2,000 km, because we need about six to seven observations in one wavelength to describe the wave pattern correctly to the first degree of approximation.

We spend some time on these elementary facts because researchers quite often make uncritical use of meteorological data in research without realizing the limited space-and-time resolution that they have. Having this concept, however, helps us to realize that certain phenomena can be treated in an explicit way; phenomena on a smaller scale necessarily have to be treated in a different way using other — more or less empirical — theories. For example, the limited density of the standard meteorological network — although never dense enough to satisfy everybody — rests upon the assumption that the smaller scale motion has little, if any, influence on the larger scale. This assumption also may be expressed in the form that we work in the Richardson and Taylor turbulence regime where the energy cascade goes from the larger scales to the smaller scale.

When we want to establish estimates of transport processes in the atmosphere there is naturally nothing better than establishing a network that is as good as we can afford. The ultimate limit is necessarily always economic in nature. Having established

the network we must observe the physical parameters that are of importance for the problem under consideration: usually pressure, density and temperature, and the "three" components of the wind, realizing that the vertical component always is difficult, if not impossible, to observe directly. In addition to the parameters that are important in describing the physical state of the atmosphere, we must naturally also observe the constituent that is characteristic for the specific problem in question. For example, if we are interested in the transport of air pollutants or pollen we must observe the concentration in our observing stations.

If for a moment, and for simplicity, we assume that we have a network dense enough to neglect the subscale, we can easily write an equation that will describe the local change of the quantity in which we are interested. Suppose that the concentration is C. The local change of C is then determined by the equation[1]

$$\frac{\partial (C\rho)}{\partial t} = - \nabla \cdot (C \rho \vec{v}) + S , \qquad \text{I}$$

which says that the change observed in a given point is determined partly by the convergence of the transport vector, $\rho C \vec{v}$, and partly by the source function, S, which also may be called the local production. Equation I can be used to describe the observed changes in a given point and especially to determine how much of the observed changes is due to transport and how much is due to local production. In order to use the equation for such diagnostic purposes it is necessary to know the three-dimensional wind vector $\vec{v}$ and the concentration in the environment. Let us furthermore emphasize that if we want to use the equation for prognostic purposes it becomes necessary to supplement the equation with a number of other equations that will predict the changes in the wind field.

If we now furthermore want to consider the more realistic case in which we have to consider our observations as mean values over a certain time it becomes necessary to go into the empirical theory for diffusion. It is naturally not the idea in a lecture of this nature to account for the diffusion theory, but let me remind you that the formal diffusion equation easily is obtained from Equation I by dividing each scalar quantity into its time mean value over a given period of time.

$$\bar{a} = \frac{1}{T} \int_{t - 1/2\,T}^{t + 1/2\,T} a \, dt \qquad \text{II}$$

and the deviation from the time mean, denoted a', i.e., $a = \bar{a} + a'$ with $\overline{a'} = o$. Using this concept we may transform Equation I into the following equation:

$$\frac{\partial \bar{C}}{\partial t} = - \vec{\bar{v}} \cdot \nabla \bar{C} + \frac{1}{\rho} \nabla \cdot \left[\rho \; \vec{K} \cdot \nabla \bar{C} \right] + \bar{S} \qquad \text{III}$$

in which the term on the left side of the equation is the local rate of change of the mean "concentration," while the first term on the right side of the equation is the advection of the mean concentration by the three-dimensional wind, the second term is the diffusion described by the diffusion coefficient $\vec{K} = (K_x, K_y, K_z)$ and $\bar{S}$ is the mean source function. Note that Equation III is a modified, empirical equation because its formulation depends upon the so-called Prandtl mixing length theory in which it is assumed that the vertical transport, $\overline{c' w'}$, can be expressed as

$$\overline{C' \; w'} = - K_z \frac{\partial \bar{C}}{\partial Z} . \qquad \text{IV}$$

In other words, vertical turbulent transfer is proportional to the vertical gradient of the mean concentration.

When we replace the instantaneous values of the parameters by the mean values and by deviation from the mean value we get the additional terms of the form, $\overline{c' w'}$, called the Reynold stresses. The information which is lost in the exchange coefficients, K_x, K_y, and K_z.

Equation III and similar equations have been used for many purposes in meteorology. They are the well known equations that are used in studies of diffusion of heat and water vapor, in evaporation from the surface of the ocean, in the description of the diurnal changes of temperature and moisture close to the ground, and in general studies of air mass transformations.

An equally intensive application of equations of this form is found in the broad area of air pollution. It is characteristic for most of these applications that the main emphasis is placed on the second and, sometimes, the third term on the right hand side of Equation III. This means that the equation can be treated without any knowledge of the velocity field in the atmosphere. Under such conditions it has been possible to consider diffusion of instantaneous point and line sources (under the assumption of constant diffusion coefficients), diffusion from a continuous point source, and steady state diffusion from an infinite line source. Most of the applications have been in the layer near the ground; the calculations have been made by simplifying assumptions with respect to the variations of the exchange coefficients.

On the other hand, the meteorologists interested in the large-scale motion of the atmosphere have put most emphasis upon the first term in the right side of Equation III and have evaluated the large-scale transport of several of the important atmospheric parameters.

The quantity C, introduced in the first equation, can be almost anything. If we want to evaluate the transport over a large area, however, we must have observations. The transports that are known with any certainty at all are therefore restricted to the parameters which are measured on a routine

[1] See nomenclature, p. 235.

basis. At the present time we have reasonably good estimates of the transports of heat, momentum, and moisture on an almost hemispheric basis, but we have little information about transports of contaminants in the atmosphere — pollen, spores, and other particles of interest to non-meteorologists.

We should furthermore be careful to point out that most of the transports that have been evaluated are for the horizontal motion. The vertical transports are almost impossible to obtain.

It is the purpose of the following data to give you an impression of the meteorologists' knowledge of the different kinds of transport that have been evaluated from atmospheric data. It is important to mention that it is possible, through the technique of Fourier analysis, to compute the contribution to a given transport from the different scales of the atmospheric motion. In several illustrations the transports can be distinguished from the different wave numbers. A given transport can be written in the form:

$$T = \sum_{n=1}^{N} T_n \qquad V$$

in which T is the total transport and all the T_n's are transports by the waves of a given wave number, n. The small wave numbers correspond to the largest wavelength. The expression, n = 1, corresponds to one wavelength around the earth at a given latitude; n = 2 corresponds to two complete waves around the earth at a given latitude, etc.

Transport of sensible heat from the ground to the top of the atmosphere as a function of latitude is shown in Figure 1. The curves are mean values for different months. The main result of the calculations is that heat is everywhere transported from south to north in the northern hemisphere, with larger amounts in the winter and smaller amounts in the summer.

A more detailed picture of the same heat transport is shown for January 1963 in Figure 2 and for April 1962 in Figure 3. Here the heat transport is shown as a function of latitude and pressure. There is pronounced maximum transport in the middle latitudes and the lower altitudes; a large percentage of the transport is carried out by the largest scales of motion.

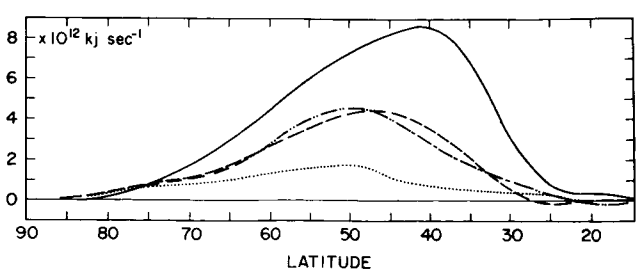


Fig. 1. Heat transport in atmosphere. Curves are mean values for different months. *Solid line* is January 1963. *Dashed curve* is April 1962. *Dash-dot curve* is October 1962. *Dotted curve* is July 1962. (kj = kilojoules).

In Figures 4 and 5 are shown a similar calculation but for July 1962 and October 1962. Notice the larger percentage of heat transport, which is carried out by the smaller scales during July.

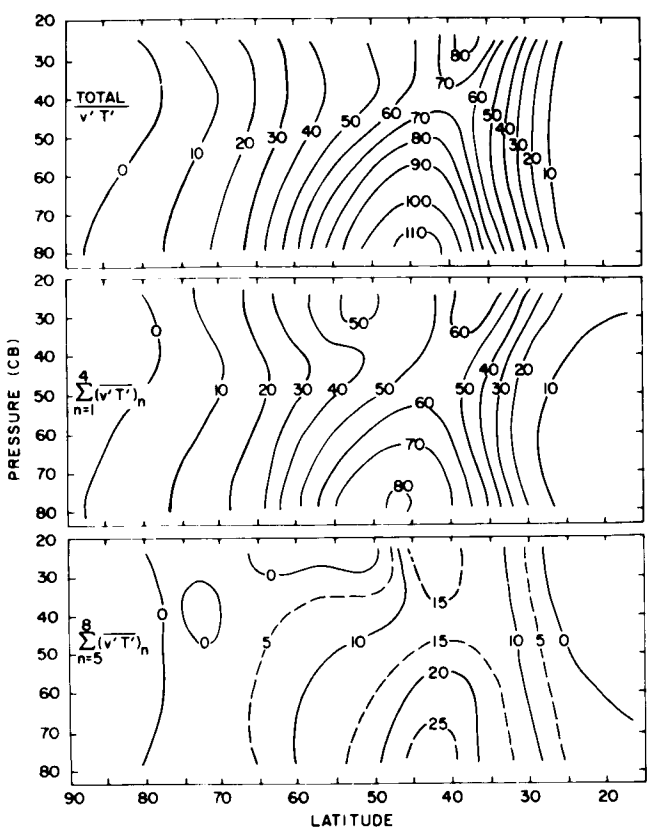
When it comes to transport of momentum in the atmosphere we find a different picture. In Figure 6 we have shown the momentum transport through the complete depth of the atmosphere as a function of latitude again for the four different months: January 1963, April 1962, July 1962, and October 1962. Note the tendency for a large northward transport in the low and middle latitudes with a tendency for negative (or southward) transports in the high latitudes. The transports of momentum are largest in winter and smallest in summer.

The more detailed picture of momentum transport for the four months is shown in Figures 7 and 8. Momentum transports are shown as functions of latitude and pressure. The maximum for momentum transport is in the high atmosphere, especially in January and to a much smaller extent in April.

The situation is much the same during the summer and fall (Figs. 9 and 10) with rather small values during the summer.

A large part of the explanation for the distribution which we have found for the momentum and heat transport is naturally to be found in the basic structure of the atmosphere. A part of the wind structure can be seen in Figures 11 and 12. These figures show the distribution of the zonal wind as a function of latitude and pressure for two different months, January 1963 and January 1962. Notice the well pronounced jet stream maximum in both months and the tendency for a secondary maximum to the north.

The cross section shown above extends only to the 200 mb level, or a little more than 10 km. The region of the stratosphere has not been investigated as thoroughly as the troposphere. It has been possible, however, to investigate the averaged distribution of temperature and zonal wind for winter and summer (Figs. 13, 14, 15, 16), temperature and wind distributions for January and July up to about 100,000 ft. Notice the temperature reversal in the layer from 10 to 20 km in the January temperature cross section in the latitude belt between equator and about 60°N. Computations indicate that although the temperature increases from the equator to 60°N in this layer, there still is heat transport to the north. In other words, the heat transport is from the cold to the warm region. This means that the cold and warm regions must be maintained by other mechanisms than in the troposphere.



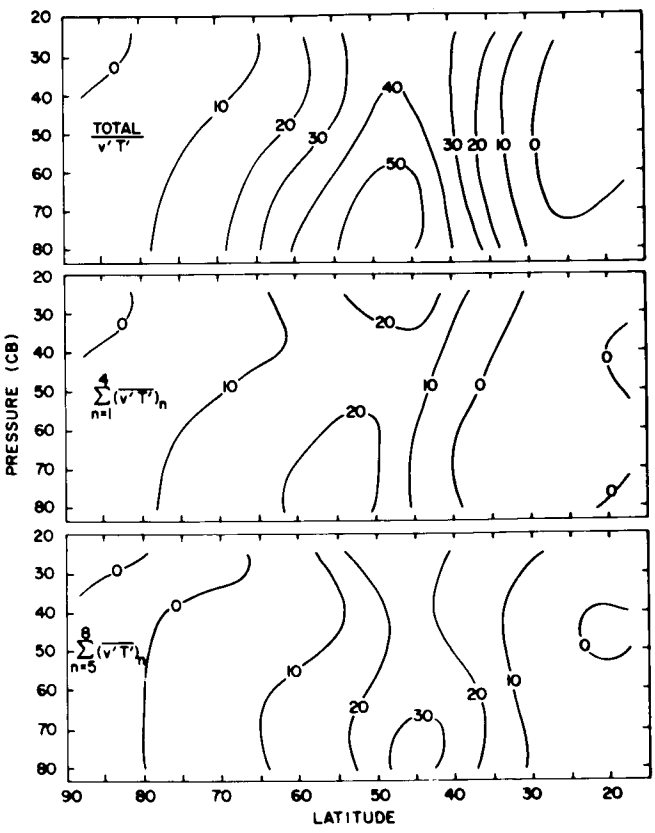


Fig. 2 (*left*). More detailed illustration of same heat transport as in Fig. 1. January 1963. *Top*, total heat transport *Center*, transports due to first four wave numbers. *Bottom*, transports due to next four wave numbers. Ordinate shows pressure in centibars (cb).

Fig. 3 (*right*). Same as Fig. 2, except April 1962.

The wind structure in the month of January in the meridional cross section shows the well developed jet stream maximum at about 40°N and at an altitude of a little more than 10 km. In the layer between 20 and 30 km, however, we find two new wind maxima: the polar night westerlies in the high latitudes and the subtropical easterlies in the lower latitudes.

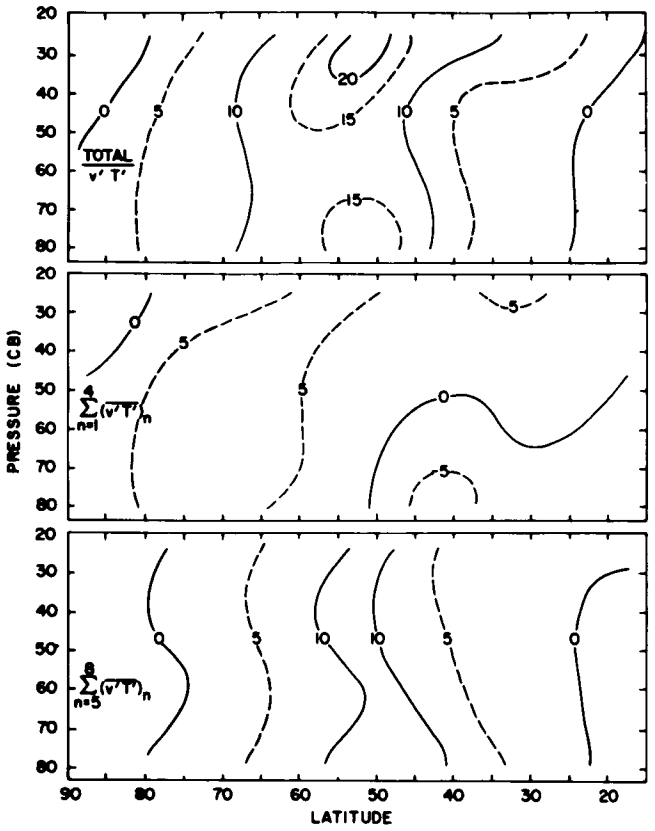
In July we find the reversal in the temperature gradient almost everywhere above 10 km with low temperatures above the equator and warmer temperatures over the poles.

In the July wind structure the tropospheric westerlies have decreased to about half of the winter values. The polar night westerlies have now been replaced by easterlies, while the subtropical easterlies still exist.

We shall next turn our attention to transport processes of water vapor in the atmosphere. Direct calculations of the transport of water vapor have been made by Starr and White and by Starr and Peixoto (Fig. 17). In the figure is shown the poleward flux of water vapor in the atmosphere as a function of latitude for the year 1950. There is a poleward transport north of about 22°N with a maximum transport around 40°N. South of 22°N there are equatorward transports with a maximum at about 10°N.

Let us denote the transport by T_w (Fig. 17). Since the curve is an average for a year, and since for such a period we might assume that we have little if any accumulation in a given latitude zone, we can write the following simple equation:

$$-\frac{\partial T_w}{a\,\partial \varphi} = P - E, \qquad \text{VI}$$



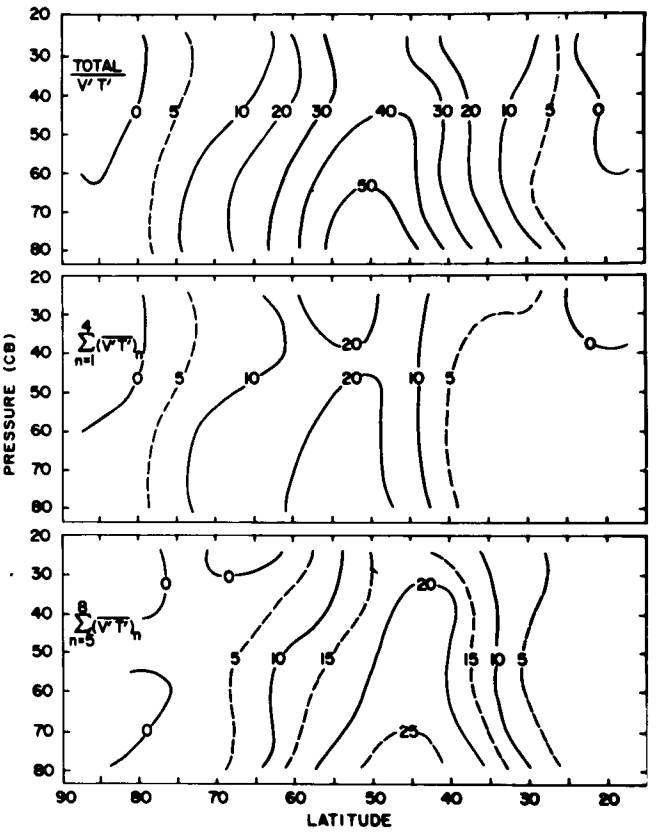


Fig. 4 *(left)*. Same as Fig. 2, except July 1962.
Fig. 5 *(right)*. Same as Fig. 2, except October 1962.

which says that the slope of the transport curve is equal to the excess of precipitation over evaporation. From the curve it is seen that north of about 40°N there is more precipitation than evaporation while there is the opposite to the south of 40°N, especially in the subtropical high pressure belt. More accurate later calculations by Starr and Peixoto have given similar results.

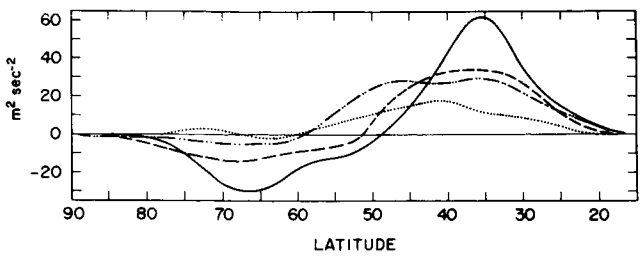
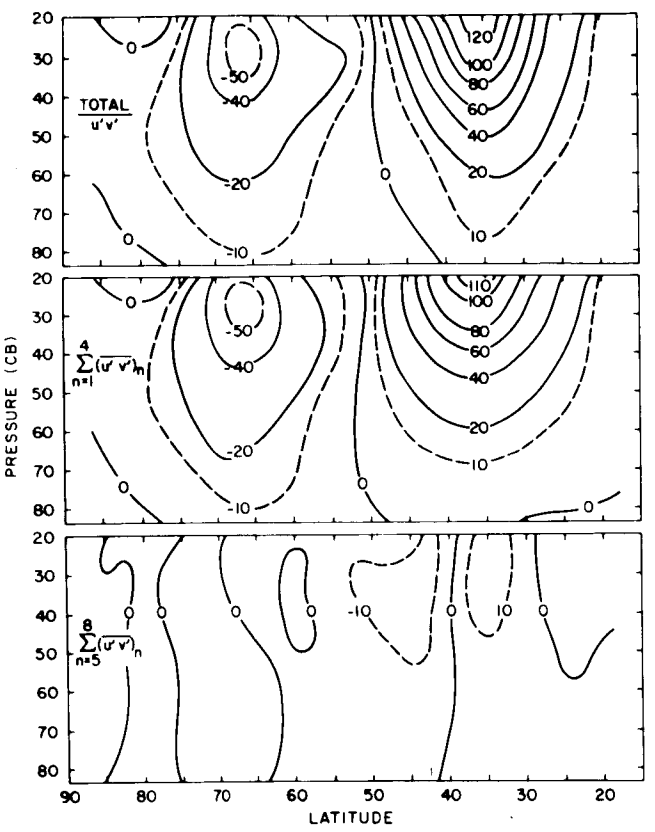


Fig. 6. Momentum transport in atmosphere. Curves are mean values for different months. *Solid line* is January 1963. *Dashed curve* is April 1962. *Dash-dot curve* is October 1962. *Dotted curve* is July 1962.

Finally, in Figure 18 one can see the moisture transport in the atmosphere of the northern hemisphere. The previous graphs have been obtained from maps of this nature by taking an average value around a certain latitude circle. Note that there are efficient transports from south to north. The centers of these are found in the low latitudes where one finds the trade winds. In the trade wind regions the moisture, so to speak, returns toward the equator in the lower parts of the atmosphere.

Unfortunately, it has only been possible to present a few of the many observational results which we now have on transport processes in the atmosphere. For many purposes it is furthermore necessary to get something better than the meridional transports that have been presented in this lecture. Remember, however, that the zonal averages were obtained by evaluating the local values of the transports, which then were averaged with respect to longitude and time afterward. It is therefore only for purposes of demonstration that we have been taking the different mean values.



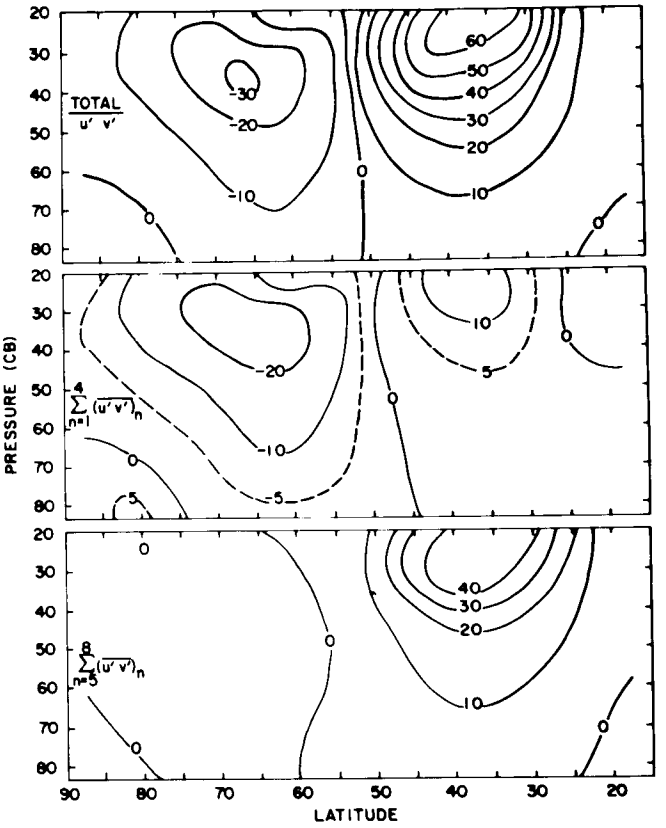


Fig. 7 *(left)*. More detailed illustration of same momentum transport as in Fig. 6. January 1963. *Top*, total momentum transport. *Center*, transports due to first four wave numbers. *Bottom*, transports due to next four wave numbers.
Fig. 8 *(right)*. Same as Fig. 6, except April 1962.

Summary

The transport processes have so far only been evaluated for momentum, heat, and moisture in great detail. Rudimentary calculations have been made for such quantities as ozone and certain radioactive tracers; we do not have detailed knowledge of these transports.

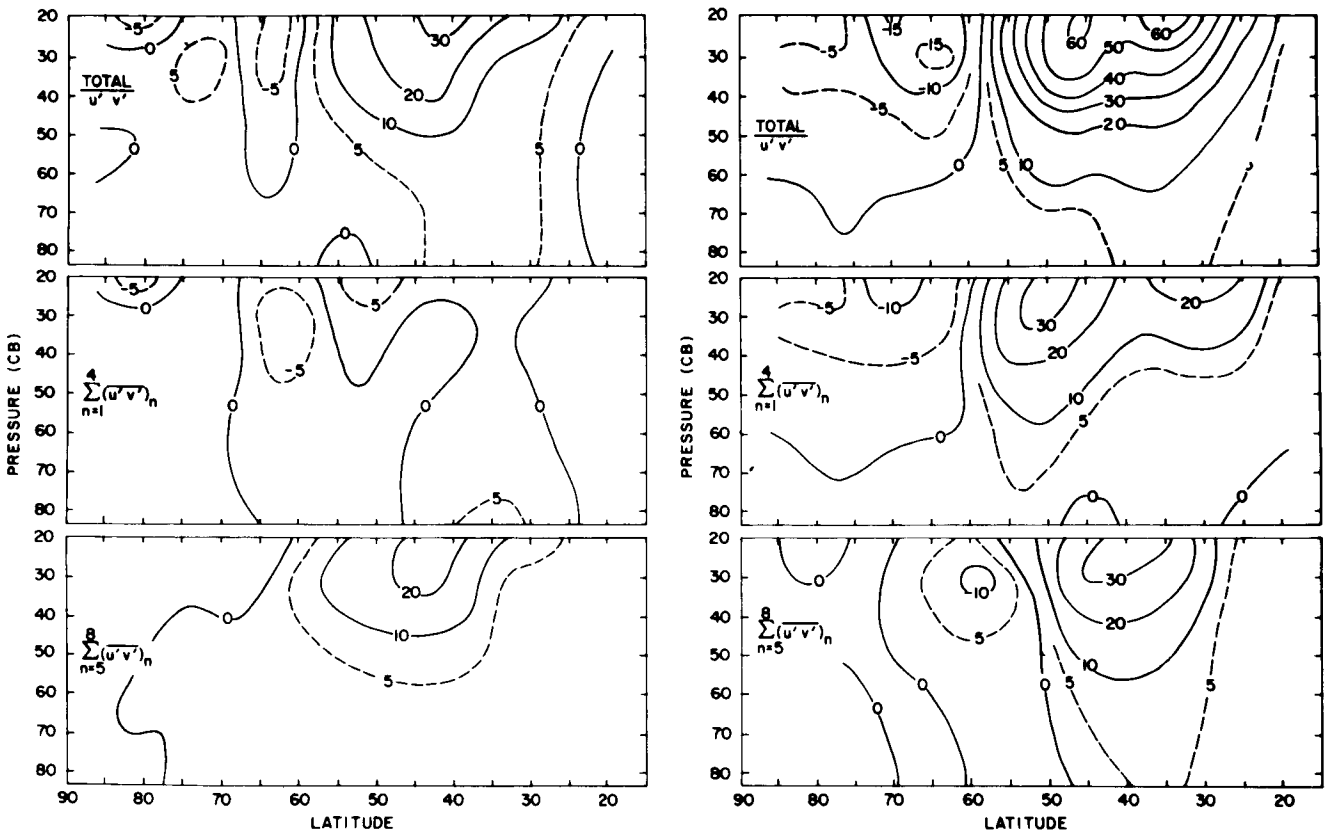


Fig. 9 *(left)*. Same as Fig. 6, except July 1962.
Fig. 10 *(right)*. Same as Fig. 6, except October 1962.

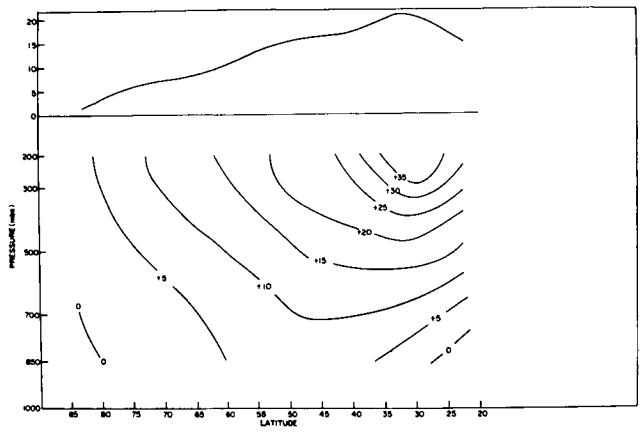


Fig. 11. Distribution of zonal wind, January 1963.

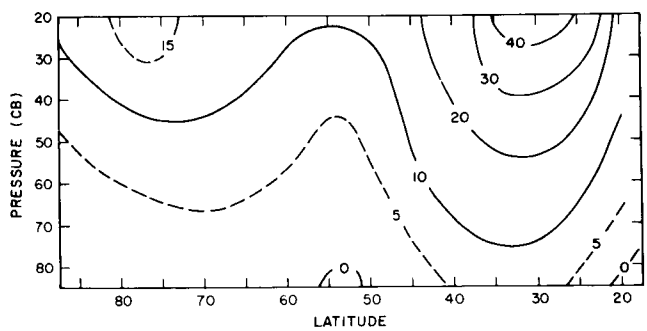


Fig. 12. Distribution of zonal wind, January 1962.

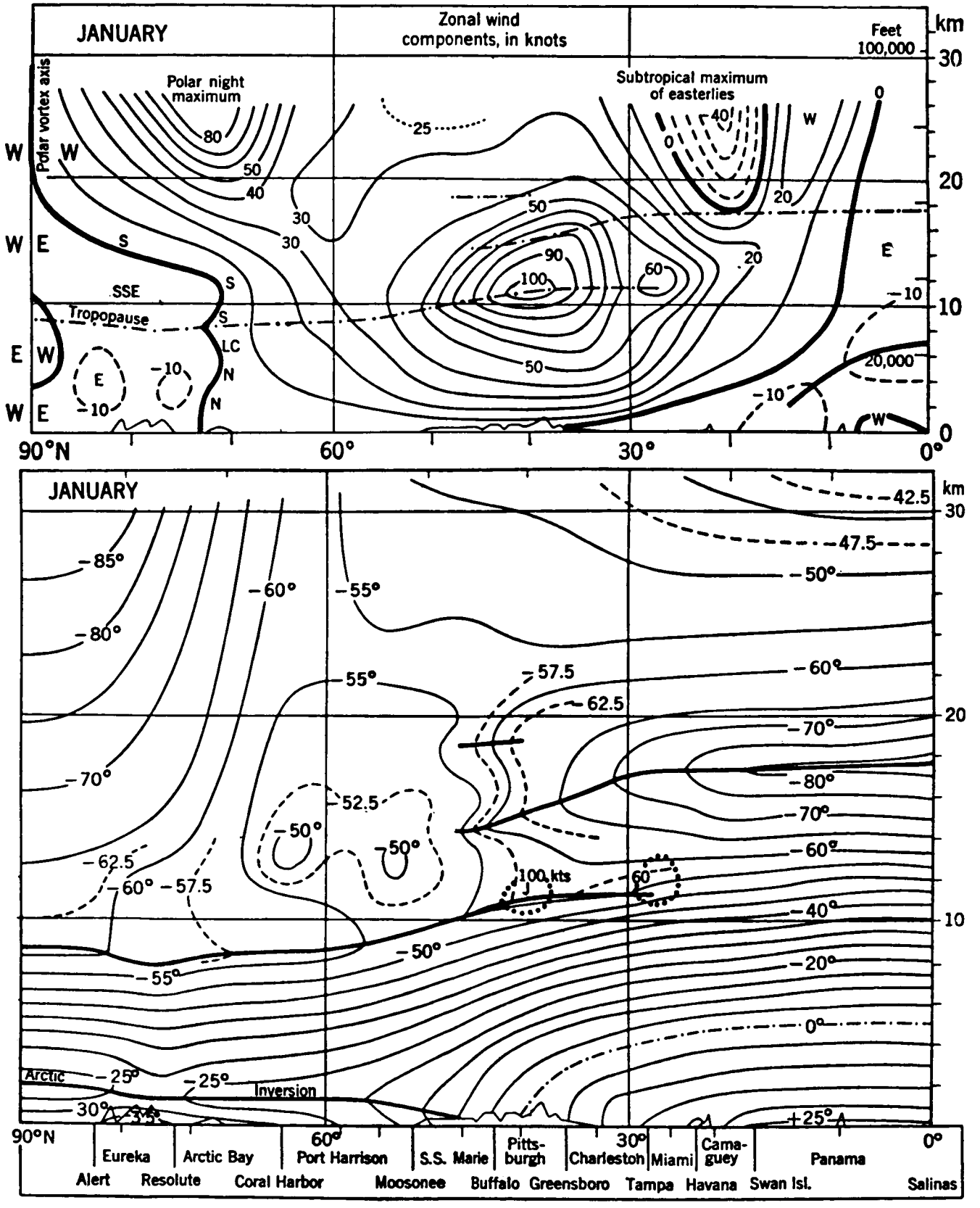


Fig. 13. *(Top)* Mean-geostrophic-wind profile along 80° W longitude for January.
Fig. 14. *(Bottom)* Mean-temperature profile along 80° W longitude for January.

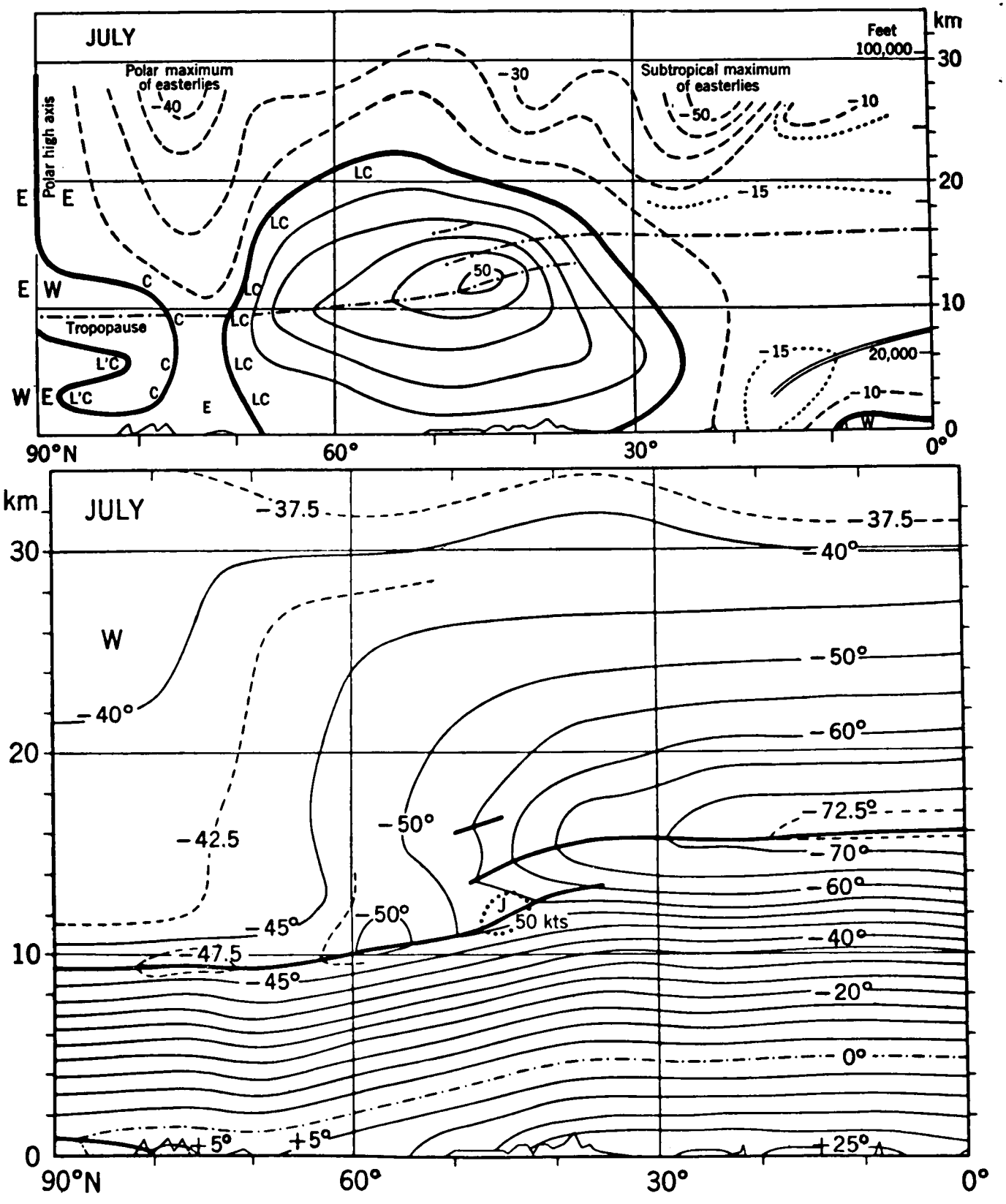


Fig. 15. (*Top*) Mean-geostrophic-wind profile along 80° W longitude for July.
Fig. 16. (*Bottom*) Mean-temperature profile along 80° W longitude for July.

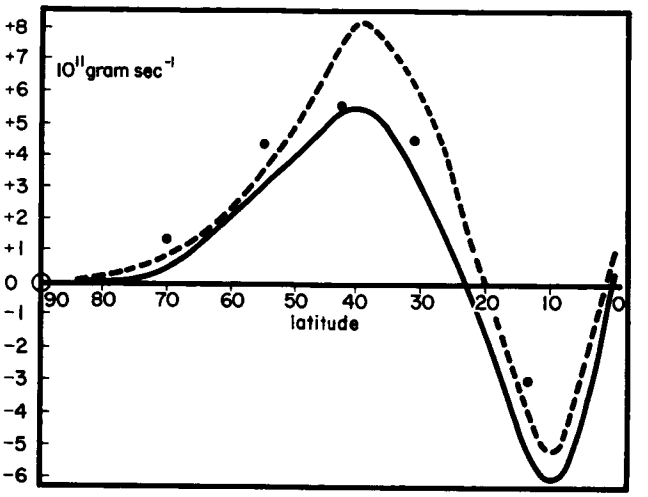


Fig. 17. Meridional distribution of poleward water vapor flux in atmosphere. *Dashed curve* and *solid curve* represent estimates of this flux deduced from evaporation and precipitation by Conrad (1936) and Benton (1953), respectively, both after data compiled by Wust (1922). *Dots* represent flux computed from atmospheric data for 1950. Units are 10^{11} g sec^{-1}.

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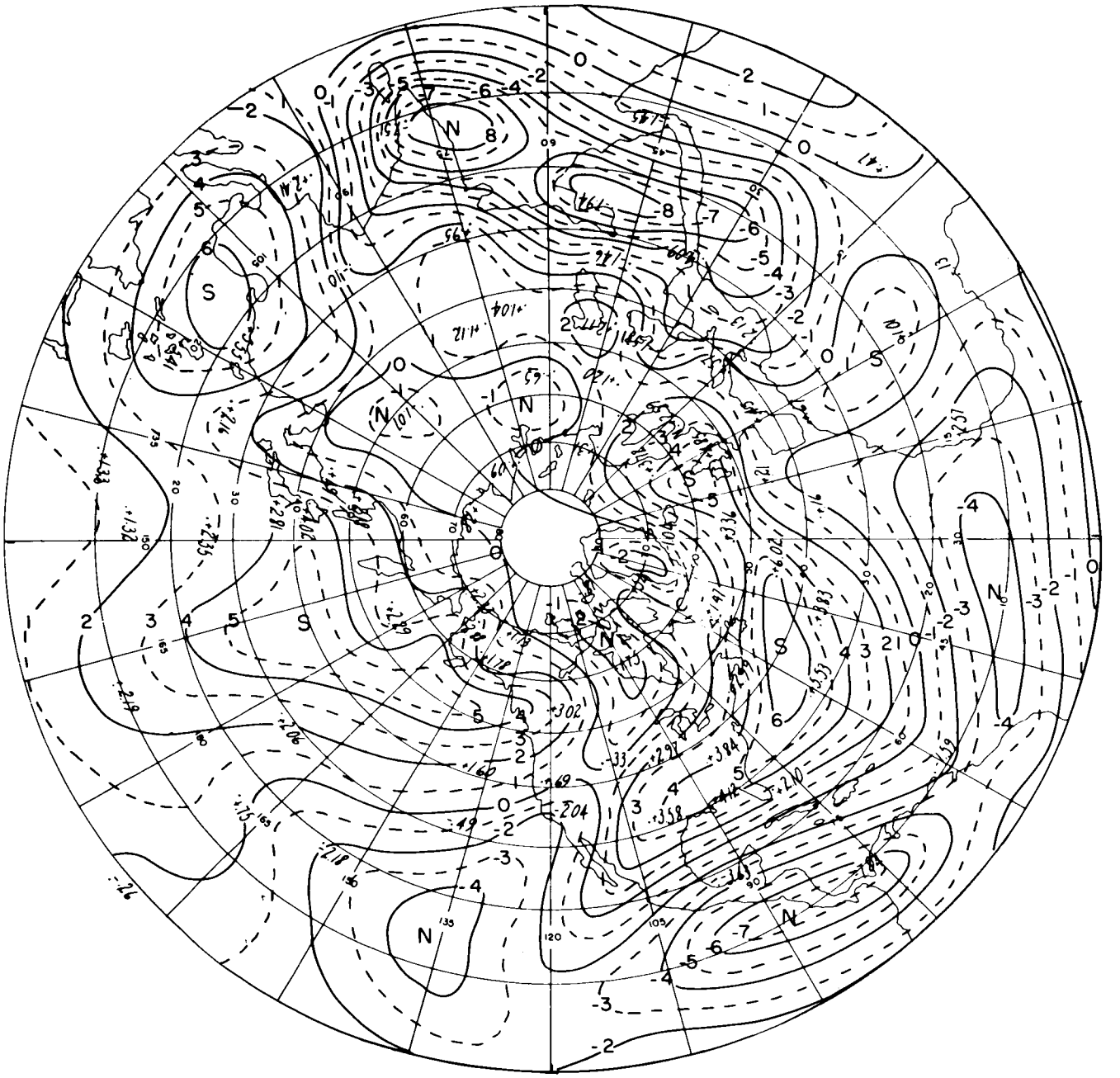

Fig. 18. Time average of vertically integrated meridional transport of moisture in 10^2 g cm^{-1} sec^{-1} for 1950. S denotes transport from south to north.

Nomenclature

C	Concentration
$\overline{C}$	Average mean concentration
$\overline{C}'$	Deviation from mean concentration
ρ	Density of air
$\vec{v}$	3-Dimensional velocity, components: u, v, and w
S	Source function
t	Time
$\nabla\cdot$	3-Dimensional divergence
$\bar{a}$	Time average
a'	Deviation from time average
$\vec{K}$	Vector diffusion coefficient, components, K_x, K_y, and K_z
T	Transport
T_n	Transport by wave-number n
P	Precipitation
E	Evaporation
φ	Latitude
kj	Kilojoule

Discussion

MacLeod — Can you expect the same type of transport that you are talking about for momentum for particles, or should we look for some kind of unique transport mechanism for air-borne particles?

Wiin-Nielsen — You cannot expect the same for particles as you can for heat and momentum. The reason is that the momentum, the heat, the density, the moisture are what we might call active parts of the atmospheric dynamics. They participate in the circulation, the thermodynamics, and the hydrodynamics of the atmosphere. If you change the temperature one place you change the wind field, the pressure field, and so on. Therefore, the parameters, mentioned above, are characteristic for the dynamics of the atmosphere. If you want to find in which way particles are moved around in the atmosphere, I suppose in many cases you will get into quantities that do not take an active part in the whole circulation of the atmosphere. Thus, we might expect the transport to be different.

I might elaborate on this. Transport calculations have been made for at least some of the radioactive tracers like rhodium. Most of the results are still under discussion. The calculations are based on the fact that we expect the tracer to diffuse according to simple diffusion laws. Those of us who have worked with heat transport and momentum transport are becoming afraid of calculations of this nature. We assume from the beginning that our tracer, our particle, is always going to move down the gradient; we now have at least a couple examples which indicate that this is not necessarily true.

Greene — Assuming that by some unknown mechanism we do get particles above the tropopause, is there enough turbulence above 30,000 ft to keep these particles suspended for more or less long periods of time (for months or years) or would Stokes' law prevail? Would the particles start falling out? Is there nothing to keep them up there?

Wiin-Nielsen — If from Stokes' law we evaluate the time it would take to get from, let us say 60,000 to 70,000 ft and down, we find that it would take a very long time. If we consider the empirical results of the particles which we happen to have put into the stratosphere, it seems that we are able to keep them in the stratosphere for quite a long time. The characteristic residence time in the stratosphere is really of the order of a month, if not a year or more. On the other hand, if we place particles in the troposphere where the overturning is more violent, the characteristic residence time in the troposphere would be somewhere from a month down to days.

Junge — I have a question about transport by various wave numbers. You showed and indicated that during the winter most transport is done by the long wave numbers. Do these long wave numbers include the standing eddies as well, or is there any separation between the transient and standing eddies as some people think?

Wiin-Nielsen — In my own heat and momentum calculations I have not distinguished between standing eddies and transient eddies.

Maybe I should first try to explain what we mean by standing eddies and by transient eddies. By a standing eddy we mean this: if you are interested in the month of January and you have 60 maps, two a day, and you want to make some transport calculation, then you would evaluate the transport each day and then you would average. It's tempting to say, "It's a lot easier just to average the maps and then evaluate the transport on that mean map." As you can clearly see you can't reverse the order. The transport on the mean map is not necessarily the same as the mean transport from all the maps. You can say, however, that the transport you would have on the mean map is the kind of feature which is outstanding. This feature controls the averaging procedure; this is normally what we call the standing eddy, the one you can see when you have averaged all your maps. The rest is then called the transient eddy. The group directed by Professor Starr at Massachusetts Institute of Technology has done a lot of work in this field. Among other things the group has differentiated between standing and transient eddies. In some months of the year they find about an equal amount in the standing eddy as in the transient. The point is that it can be terribly misleading to evaluate the transport from the mean distribution of the wind field or the temperature field.

The thing I am saying is that you can't get lazy about this. The only thing which really counts is to evaluate the transport quantities in as extensive a network of space and time as you can. Don't average everything out. If you do average out you will find at least in some cases that the mean waves (the so-called standing eddies) transport in the other direction because of averaging out certain parts of your motion.

Brown — You mentioned that the fact that you have relatively few stations in certain parts of the world limits the resolution of your network. Is the main difficulty the oceans where you have few stations, or is it the countries behind the Iron Curtain where communication is deficient?

Wiin-Nielsen — The main difficulty is over the ocean.

Brown — Would you care to comment on the suggestion that one might overcome this by using floating stations, not on ships, but on buoys, and by acquiring the data by satellites?

Wiin-Nielsen — I'm aware of one system which is under consideration at the present time. The system makes use of a satellite, which I will comment on a little later. There have been serious suggestions that it would be possible to use large numbers of essentially constant-pressure or constant-density balloons. They would then float around in the atmosphere at a designated level. Instruments thereon would record temperature and pressure. Observation of the movement of the balloon, of course, would give the wind field. If I understand this system correctly, each of these balloons would have a transmitter that would send information first to a satellite, and then down to one or more collecting stations. As far as I can judge, the system looks promising and economically feasible; I wouldn't be surprised if this is what we are going to have 5 to 10 years from now. There are, of course, a number of questions. How long would the balloons stay up? Would there be a tendency for these balloons, once they are released, to run together (because they are also particles and they are also being transported)? Would they end up in long lines? Would they all collect in the jet stream? If they collect in the jet stream it wouldn't be too bad because that's the region of largest variance and the region in which one wants to make lots of observations. In addition, there is simply a mechanical problem of having the particles stable and having them stay in the atmosphere.

Steinberg — Dr. Mantis indicated that the water vapor in the atmosphere decreases through the troposphere up to the tropopause, and then starts to increase again as in the mixing ratio. Have your studies indicated how water can be transported to the stratosphere to give us an increase in mixing ratio?

Wiin-Nielsen — We have agreed in our studies as to the distribution of water in the vertical direction as far as the tropospheric values are concerned. As a matter of fact, when you come up to about 5 to 6 km you have really covered most of the water. Only information from the troposphere has gone into my illustrations. We have not tried at any time to evaluate the transport of water in the stratosphere, so I don't know. The only thing which I should like to comment on in this respect is that in some of the vertical calculations where people have been trying to, let's say, explain the distribution of temperature in the atmosphere, temperature has been the most important question. If the water vapor pressure increases in the stratosphere, how much water is there? If one tries to account for the temperature distribution he finds that it is extremely sensitive to the amount of water in the stratosphere. As a matter of fact, one can change the temperature in the stratosphere considerably by putting in a few extra grams per kilogram of water. Although your question is most pertinent I don't have the answer.

Extrusion of Stratospheric Air Into Troposphere

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Abstract

Contaminants that have a negligible settling rate are transported by the mean flow and diffused by the small-scale eddies (turbulent deviations from the mean) after they are injected into the atmosphere. The diffusion is not symmetrical because the diffusion coefficients vary with direction and because the concentration gradients are changed by the mean flow. The latter effect, often overlooked or underestimated in diffusion studies, can be demonstrated by three-dimensional trajectory analyses and radioactivity observations. Effect is important when mass is transported from stratosphere to troposphere and it is also important when contaminants are injected into and transported in the troposphere.

One year ago today, B50 and B57 aircraft took off from airfields in California and New Mexico to conduct the first mission of Project Springfield. This project, sponsored by the Defense Atomic Support Agency, the Atomic Energy Commission, and the United States Weather Bureau, was organized to verify, by radioactivity measurements, a theory of stratospheric-tropospheric mass exchange. The stratosphere contains the major burden of radioactive particles produced by the nuclear bombs. It is important to know how this debris leaves the stratosphere; at what rate and concentration.

The theory to be tested predicts the extrusion of radioactive layers from the stratosphere into the troposphere. It also emphasizes transport rather than diffusion as the dominant mode of exchange.

If the theory were proven correct it would challenge the accepted concepts of radioactive fallout, clarify the roles of transport and diffusion in the production of both wet and dry fallout, and also lend credence to a special method of atmospheric trajectory analysis.

The B50's, more specifically the WB50 weather reconnaisance aircraft, were equipped to sample the radioactivity by capturing the radioactive particles on filter papers exposed to the air stream. Both discrete and continuous samples were taken. After a 20-minute exposure the discrete samples were withdrawn and sealed. Upon completion of the flight they were sent to laboratories for isotopic analyses. The filter paper continuously exposed to the air flow was backed by a Geiger counter. Its counting rate was recorded on a strip chart from the time of takeoff to landing.

Of course, the WB50's were also equipped to make complete meterological observations which included temperature, dew point, wind direction and speed, cloud types, bases and tops, and subjective estimates of the intensity of turbulence. All measurements were made and recorded every 10 minutes by an experienced weather observer.

It was his responsibility to study these data during the flight to determine when the aircraft traversed a layer of the type predicted by the theory. If a layer was detected and its boundaries determined he was directed to initiate a predetermined flight pattern to obtain filter samples.

As director of the project it was my responsibility to try to predict weather patterns favorable for layer formation and then to select the optimum flight tracks and altitudes for the aircraft. To assure availability of aircraft in a large-scale project of this type, an alert was issued 72 hours in advance of a mission. This was followed by 48-hour and 24-hour communications. To assist the observers and pilots I issued a detailed forecast just before takeoff. The forecast included the location of the layer, its thickness, wind shears, and temperature gradients. Using the predictions as a guide they began their search.

The B50's were more capable of detecting and monitoring the layers so their flights were restricted to the troposphere where layers were expected. The B57's were flown above them to obtain samples from the stratosphere and upper troposphere. They were assigned flight tracks perpendicular to the axis of the jet stream that they located by monitoring their drift angle. Their sampling positions were then determined relative to the jet core.

Before we discuss the flight and sampling data let us establish a visual context through a sequence of schematic diagrams. We shall view the layer formation in two dimensions by making a vertical cut through the atmosphere perpendicular to the wind flow. The upper left diagram in Figure 1 depicts a simple initial state of the atmosphere. The stratosphere is toned gray, the troposphere is white, and the heavy line separating them is the tropopause. The thin lines that slope upward to the north in the troposphere and downward to the north in the stratosphere are isolines of potential temperature. The vertical spacing between these lines forms the basis for distinguishing between the stratosphere and troposphere.

The air parcels along a particular theta line do not actually have the same temperature, but each parcel has the potential to attain the same temperature if compressed to the same reference pressure. For example, an air parcel at 500 mb with a temperature of -27 C will warm to +27 C if compressed to 1,000 mb without gain or loss of heat. We could therefore label this parcel with an θ= +27 C or θ= 300 K even though its actual temperature is 246 K.

Thus we see that if no heat is added or subtracted from a moving parcel it conserves its value of theta; it is in this sense tagged. Since dry air is a poor radiator and conductor we can assume, as a first approximation, that its theta is conserved. In Figure 1 the theta lines are not labeled, but if they were, the values would increase to the south in the troposphere and increase with height everywhere.

As I mentioned, the distinction between the stratosphere and the troposphere is based essentially upon the vertical separation between consecutive theta lines. Where these lines are closely spaced in the vertical, the stability is high; where the lines are widely spaced in the vertical, the stability is low. This refers to a parcel stability and tells one about the tendency for bouyancy forces to return a parcel to its initial position if it is displaced vertically.

Now let us consider the formation of the layer and the accompanying changes in the theta lines. In Figure 1 is shown how the stratosphere and troposphere can change from a simple distribution into a more complex distribution, one in which the temperature gradients, that is, the horizontal and vertical spacing of the theta lines varies, and the characteristic shape of the tropopause varies. Proceeding from the upper left, to upper right, to lower left we notice a steepening, and then a folding of the tropopause. As the tropopause folds, the stratospheric air in the folded layer descends and moves southward along the constant theta lines.

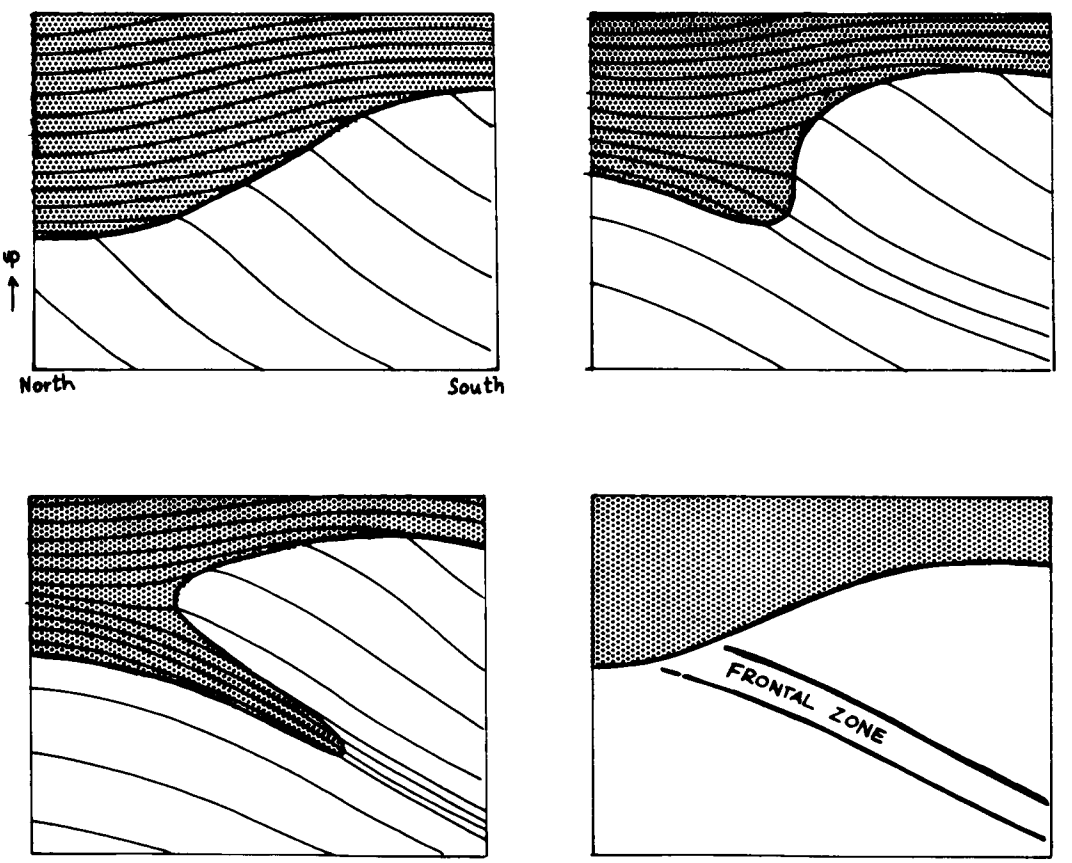


Fig. 1. Vertical cross sections showing steepening and folding of tropopause. Thin lines are potential isotherms, constant theta lines, with theta increasing upward. Heavy line is tropopause. Diagram in lower right depicts a conventional analysis of diagram in lower left.

Simultaneous with the folding, the theta lines in the troposphere change both their horizontal and vertical spacings. The tendency to concentrate is called frontogenesis and the concentration contiguous to the folded layer would be called a frontal zone. Notice that the concentration extends well into the stratospheric air. This is significant because in the frontal zone and in the folded layer the horizontal temperature gradients are similar and the stabilities are similar. If a meteorologist is not cognizant of the folding process he will assume both are but one frontal zone that extends up to a smoothed tropopause, as shown in the lower right diagram. In this case the entire frontal zone would be considered as tropospheric air.

Obviously the two lower diagrams imply drastically different distributions of stratospheric air. They also imply different distributions of radioactivity and other stratospheric tracers such as ozone. Trajectories computed on surfaces of constant theta indicate that the lower left analysis is correct. The distribution of another quasi-conservative meterological quantity called the potential vorticity also supports the lower left analysis. Direct measurements of the distribution of radioactivity will solve the problem, so let us now turn to the flight data.

On April 18, 1963 two B50's and two B57's were sent to the southwestern corner of Wyoming and assigned a southeast flight heading. The B50's were delayed over Wyoming. Because of clouds they had difficulty obtaining a clearance to cross the airways. The lower altitude aircraft aborted and the upper aircraft only had time to complete the first portion of the mission. The cross section shown in Figure 2 was prepared from the flight data and the regular radiosonde data. The analysis extends from Boise, Idaho to Midland, Texas. Lines of constant theta are drawn in black at a 2-K interval. The thin horizontal line represents the path of the B50 aircraft. (Lines of constant wind speed, drawn in red on the color slide, have been filtered for black and white reproduction.)

When the aircraft traversed the layer the wind speed increased by 85 knots, the temperature increased 20 K. Three radioactivity samples were taken. The first at the turn, the second just before re-entering the layer, and the third in the layer. The total activity of these samples expressed in units of disintegrations per minute per standard cubic foot of air (dpm/scf) was plotted at the sampling site. South of the layer the activities were low, 1.9 and 2.3 dpm/scf. In the layer the activity was high, 136 dpm/scf. The latter compares closely to the activities of the B57 samples taken in the stratosphere north of the jet which were 114, 161, and 180 dpm/scf.

In addition to these discrete samples we have the continuous record of the counting rate from the exposed filter. The record indicates uniformly high radioactivity through the entire zone and shows a rapid change to low activity in the tropospheric air. This data supports the lower left analysis of Figure 1 but leaves something to be desired, i.e., measurements at lower altittudes.

Three days later we had another chance and this time all went well. The cross section for this mission is presented in Figure 3 on a more expanded scale. The theta interval in this case is 4 K. In sympathy with the observed snow showers the clouds are indicated by dashed lines. Note that the cloud tops decrease in elevation with the layer.

Both B50's traversed the layer twice, then changed altitude and traversed it again; therefore, we have discrete samples and continuous counting rates at 19, 21.5, 24, and 26,000 ft above sea level. South of the layer the activities were close to 2 dpm/scf. In the layer the upper aircraft No. 2 obtained two samples of 92 and 88 dpm/scf. Aircraft No. 1 did not take a sample in the layer at 21,500 ft, but did at 19,000 ft. The latter activity was 20 dpm/scf.

The continuous counting rate for the first two traverses of aircraft No. 2 is shown as a heavy line in Figure 4. On the same diagram the temperatures measured every 10 minutes are connected with a thin line. Appropriate scales are on the left and right, respectively. Since the filter continually accumulated debris the counting rate was proportional to the integrated activity. Thus the activity itself, was proportional to the slope of the curve. The slope increased rapidly when the aircraft entered the layer, remained approximately constant in the layer, then decreased rapidly as the aircraft re-entered the tropospheric air. The layer was well defined by both the radioactivity and the temperature. Once again the temperature change was a big 20K. The oscillation in slope in both traces at 2210Z was not a property of the layer, rather it was due to a change in flight path relative to the layer. After re-entering the layer the pilot turned 90° left to sample upwind. The layer had changed its orientation so the aircraft approached the warm boundary again.

After two traverses the counting rate exceeded 6,000 cpm. For comparison the counts per minute for flights in tropospheric air are presented in Figure 4. Over the same time interval they increased only a few hundred counts above background.

I mentioned before, aircraft No. 1 did not sample in the layer at 21,500 ft, but a continuous record was made during flight. It is shown in Figure 5 directly beneath aircraft No. 2's record. The temperatures were warmer, as they should be at the lower altitude, and the traces did not change slopes at 220Z because aircraft No. 1 did not turn to sample; everything else was almost identical. We know, then, that the concentration at 21,500 ft was the same as at 24,000 ft. This in turn was only slightly less than the concentrations at 29,000 ft and 28,000 ft. The continuous records and the samples indicated that the stratospheric flow was not mixing with the tropospheric air between 28 and 21,000 ft. At 19,000 ft, however, the continuous record showed two tilted steps within the layer. This suggested that mixing was occurring and the sample at this level confirmed it, since its activity was only 20 dpm/scf.

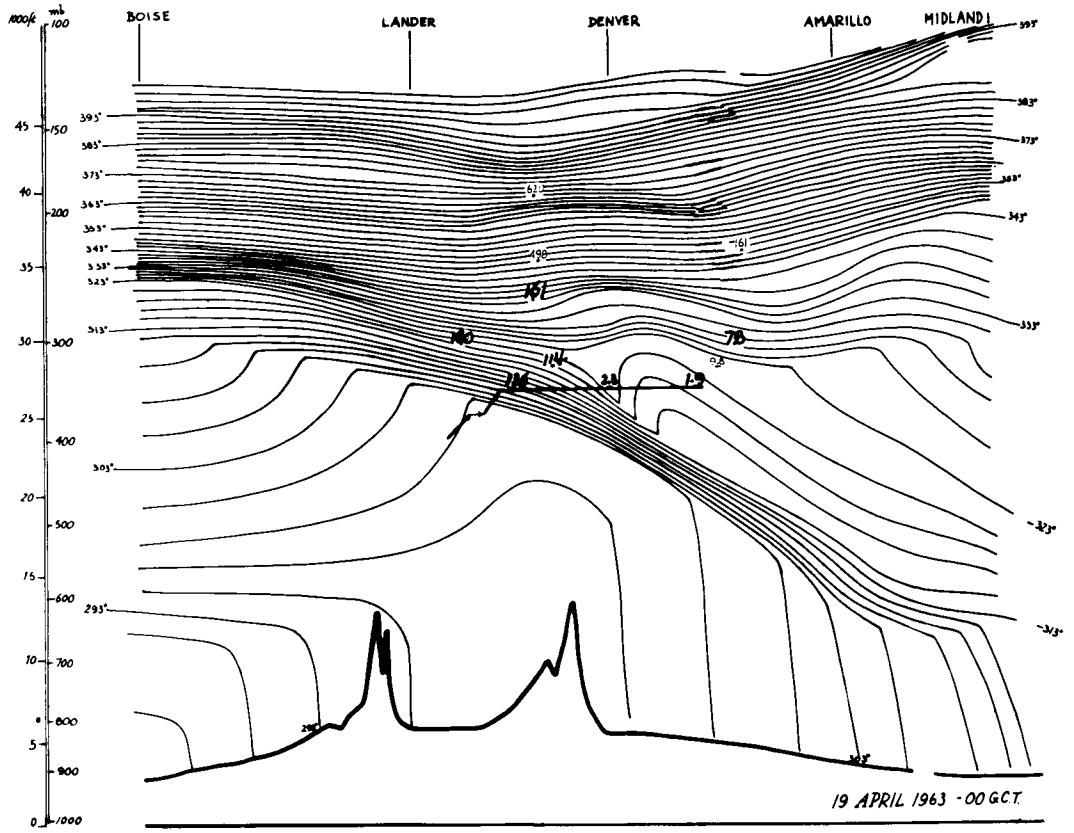


Fig. 2. Vertical cross section for April 19, 1963. Potential isotherms drawn at intervals of 2 K. Total activity at point of sampling expressed in disintegrations per minute per standard cubic foot of air.

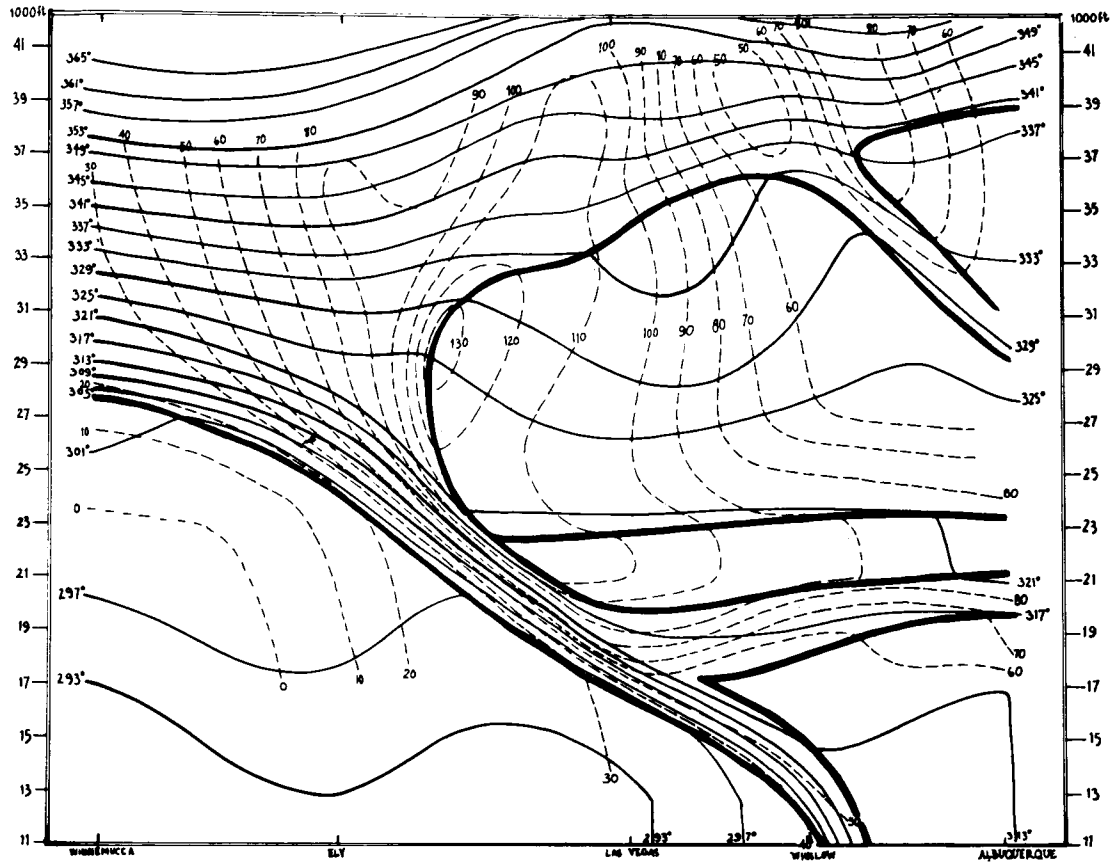
All the evidence from these two and other missions confirms the theory of tropopause folding and the extrusion of radioactive-laden air from the stratosphere into the troposphere.

The uniformity of the radioactivity in the layer and the sharp gradients at the boundaries corresponded closely to the distribution of potential vorticity. Both distributions support the view that transport rather than diffusion is the major mode of stratospheric-tropospheric exchange. The evidence of mixing also coincides with the transition from large to small potential vorticity and it suggests that the mixing is along the theta surfaces not normal to them.

We have considered the tropopause folding in two dimensions. Now I should like to convey to you the three-dimensional trajectories characteristic of the extruded stratospheric air. In Figure 6 is illustrated a family of trajectories that fans out from the stratosphere. Initially, all of the trajectories descend. The right hand branch of the flow, facing downstream, continues to descend to low elevations. As it descends the air decelerates and curves to the right. At low elevations it forms the easterly circulation to the south of a high pressure cell. This branch of the flow transports radioactivity and ozone to low elevations where turbulent mixing generated at the ground mixes the radioactivity down to the earth's surface. Therefore we can assume that this branch produces dry fallout.

The other branch, the center and left side of the flow, descends until the air reaches the trough. East of the trough its vertical velocity becomes

Fig. 3a *(top)*. Vertical cross section for April 22, 1963. Thin continuous lines are potential isotherms. Thin dashed lines describe wind speeds in knots at intervals of 10 knots. Heavy lines serve as reference lines for Figure 3b.
Fig. 3b *(bottom)*. Flight paths of B50 aircraft and total activity of B50 and B57 samples. Refer to Figure 3a.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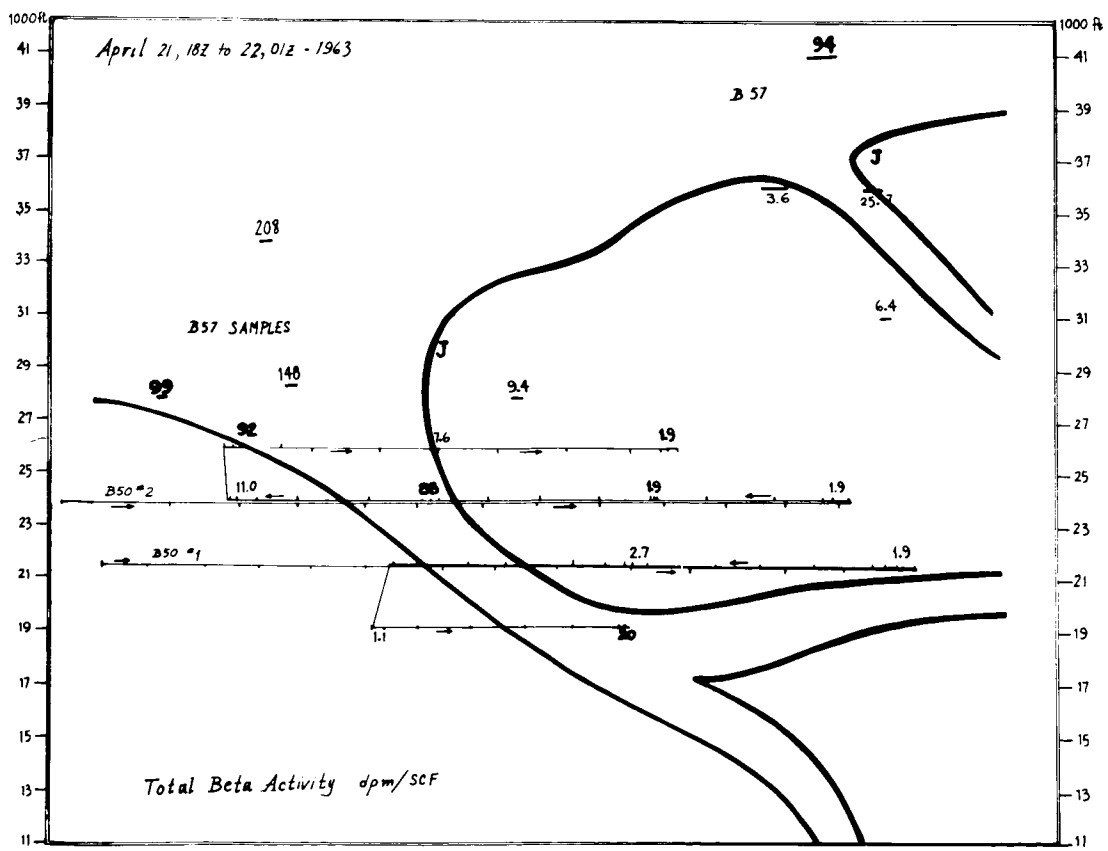
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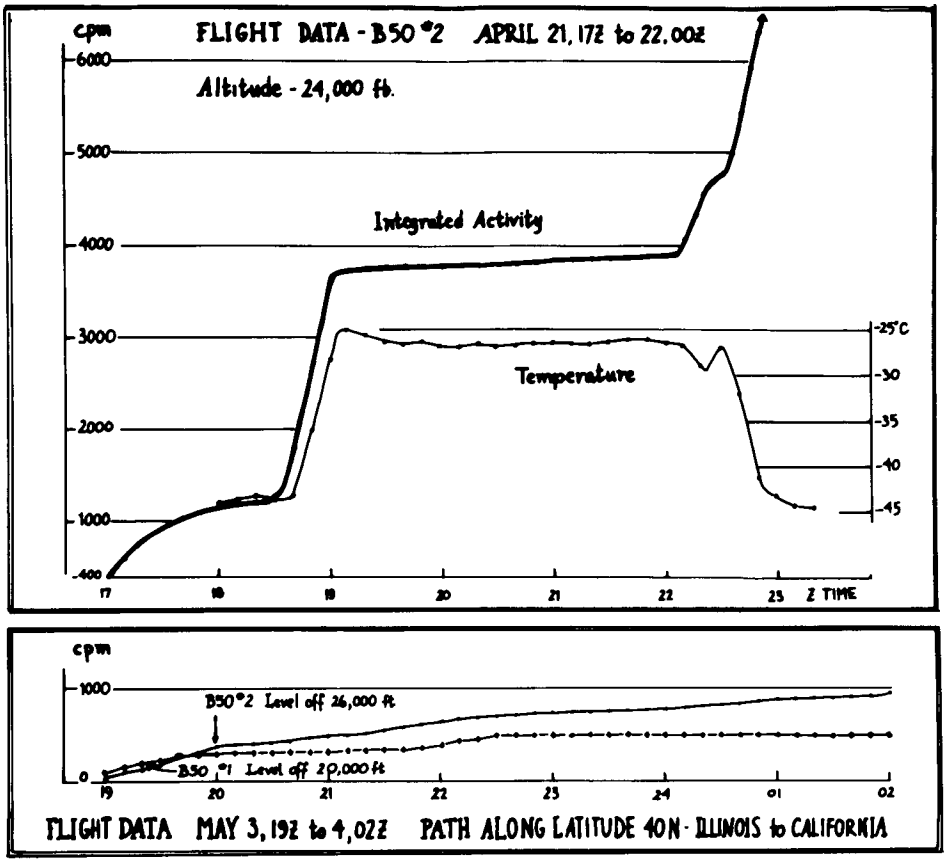


Fig. 4. Record of activity on continuously exposed filter paper and record of observed temperature at 24,000 ft on April 22, 1963. Activity is expressed in counts per minute.

positive. It ascends as a dry layer above ascending moist or saturated air. Initially the layer acts to suppress cumulus cloud development. But the stratification of dry over moist is potentially unstable and with ascending motion the instability is realized.

Suddenly, rain showers and thunderstorms develop along the leading edge of the extruded layer. The thunderstorms penetrate the layer and mix the stratospheric air into the precipitation-generating cells. By this process the radioactivity is incor-

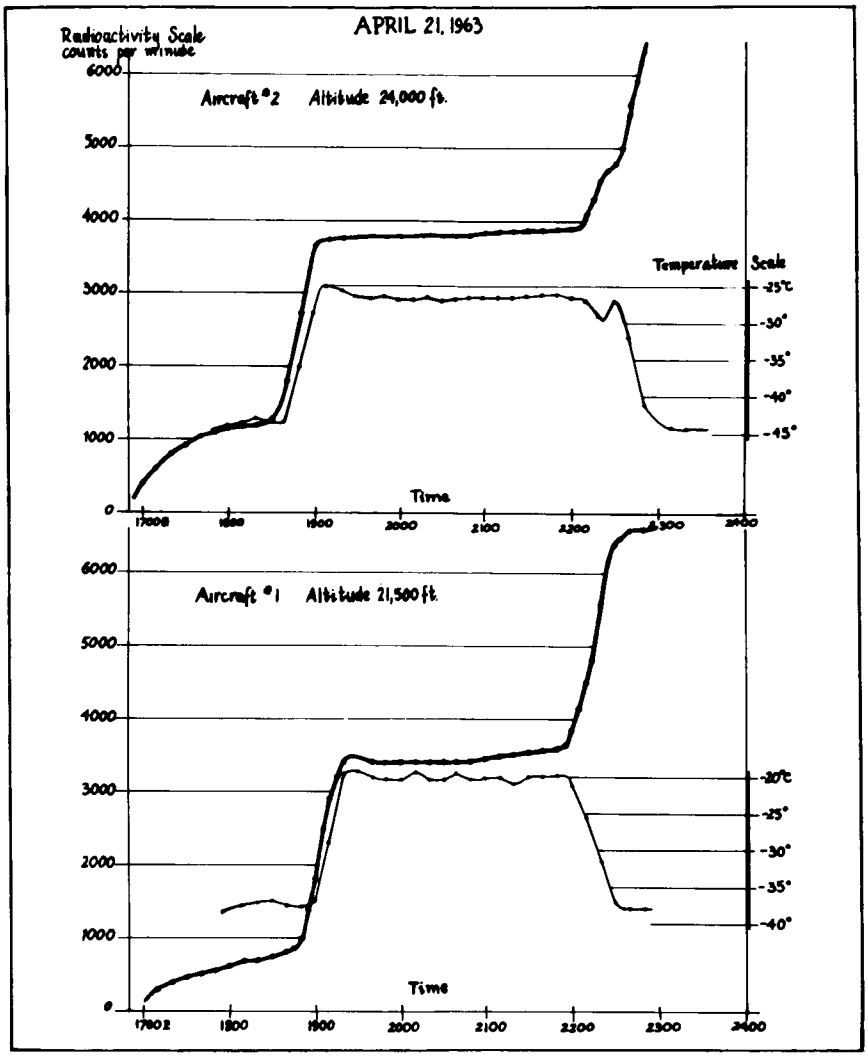


Fig. 5. Activity vs. temperature as observed by aircrafts No. 2 at 24,000 ft and No. 1 at 21,000 ft, April 22, 1963.

porated into the precipitation, and wet fallout results.

From this brief discussion we can appreciate how the stratospheric air contributes to both the dry and wet fallout. I want to emphasize that the entire process of exchange from stratosphere to ground can occur in 24 to 48 hours.

The trajectories shown in Figure 6 are typical of those computed on constant theta surfaces to the west of a developing cyclone or low pressure system. When storms develop, the increase in kinetic energy is supplied by a decrease in potential energy, or in other words, a decrease in the height of the air's center of mass. This requires a sinking of the cold air and rising of the warm air. Obviously with development the air does not travel horizontally or at constant pressure. The air sinks through the pressure surfaces west of the trough and ascends through them east of the trough. This phenomenon

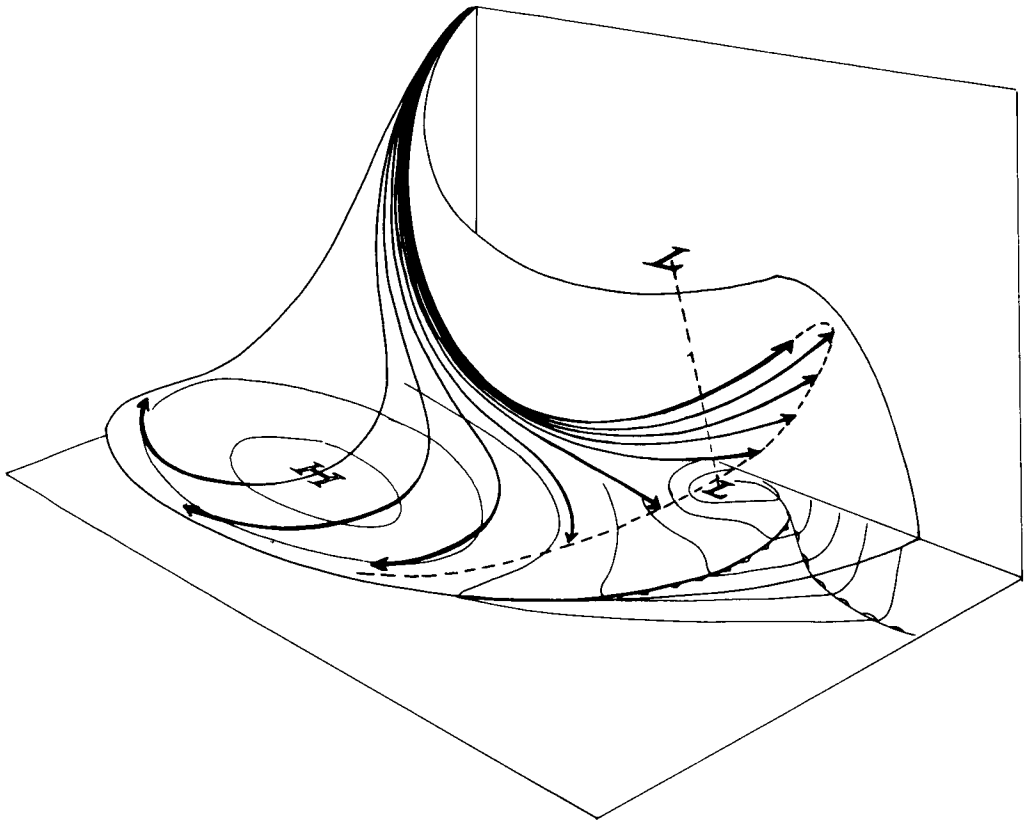


Fig. 6. Characteristic trajectories of extruded stratospheric air with reference to a surface weather map.

leads to erroneous trajectories if they are computed at constant pressure. The errors are by no means insignificant (Fig. 7). In Figure 7, the trajectory labeled "isentropic" could also be labeled "constant θ" since the entropy is proportional to theta.

We have seen how the descending motions might transport stratospheric air to low elevations. The opposite set of conditions is equally probable. In regions of warm advection the southerly flow ascends and accelerates. Within 24 to 48 hours spores or other surface air pollutants can be transported to 15 to 30,000 ft where they move rapidly eastward in the upper level jet. An example of these ascending trajectories over the southeastern United States is presented in Figure 8. In this case, the air ascending from western Cuba, *dashed line*, increased in speed from 6 m/second to 60 m/second in 24 hours. Its speed continued to increase as it shot across the north Atlantic. Over Europe it might have entered the stratosphere, but more probably it descended again in a manner similar to that shown in Figure 6.

There is also another method which may carry surface pollutants to high elevations. This is illustrated in Figure 9 by the column of dust that reached to 20,000 ft. Vigorous vertical mixing caused by intense surface heating of dry soil and a downward transport of high momentum stirs the air and carries the surface contaminants upward. Aloft, the high wind velocities then transport the dust or spores to higher elevations.

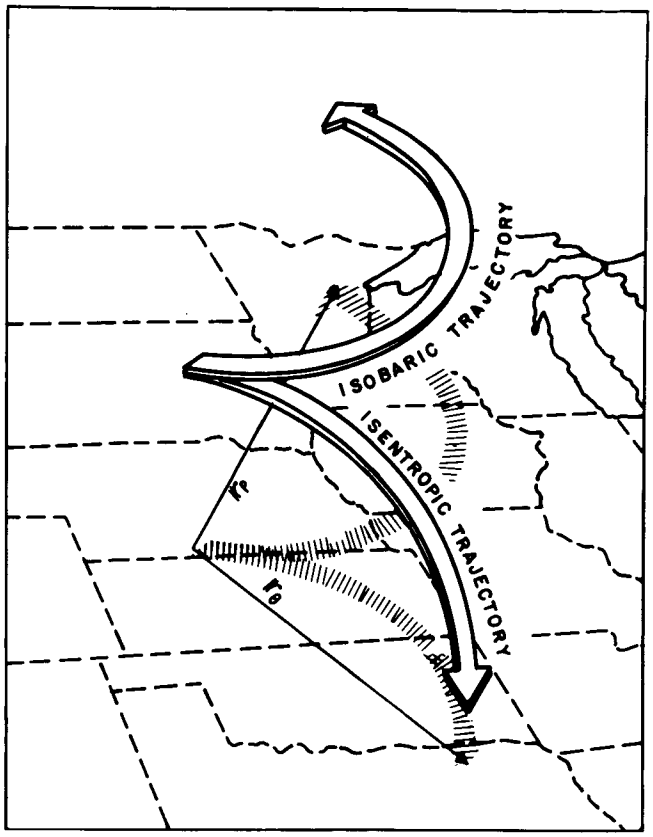


Fig. 7. Difference between a trajectory computed at constant pressure and at constant theta or entropy.

Summary

We have seen evidence of the folding and extrusion of stratospheric air. The evidence also supports the concept of a downward stratospheric-tropospheric transport rather than a diffusive mixing across the tropopause. I have also indicated how surface or near surface air can be rapidly transported to great heights and enter the stratosphere. These circulations are related to the development of the mid latitude storms and should be expected to occur with each major storm development.

Nomenclature

dpm/scf Disintegrations per minute per standard cubic foot of air

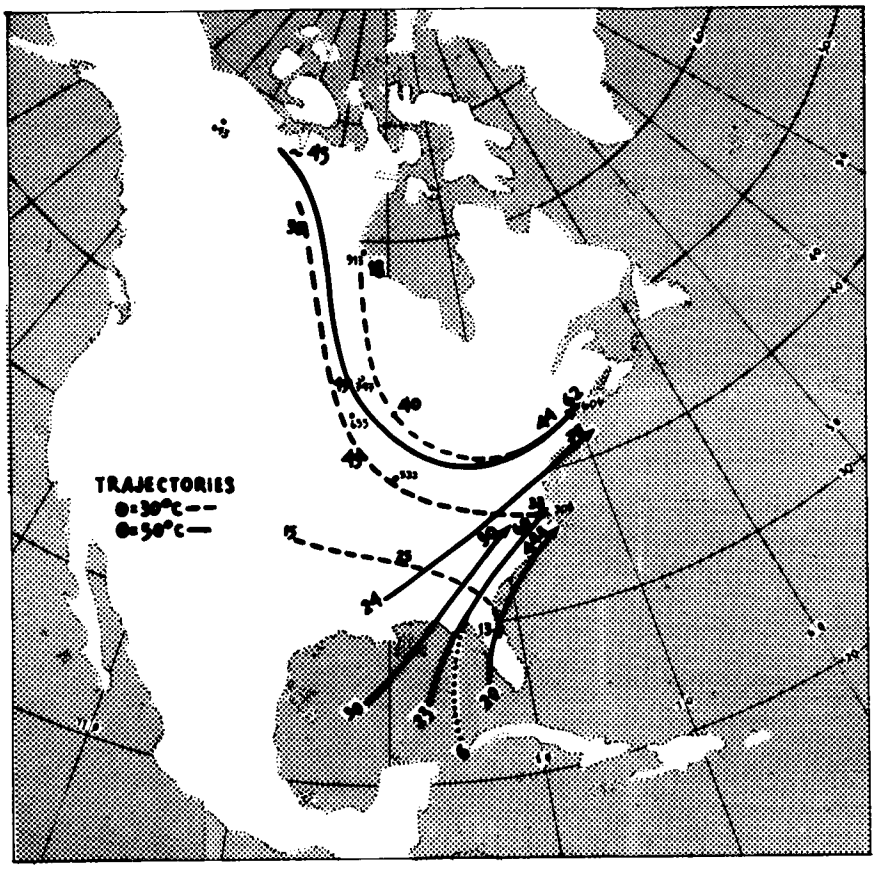
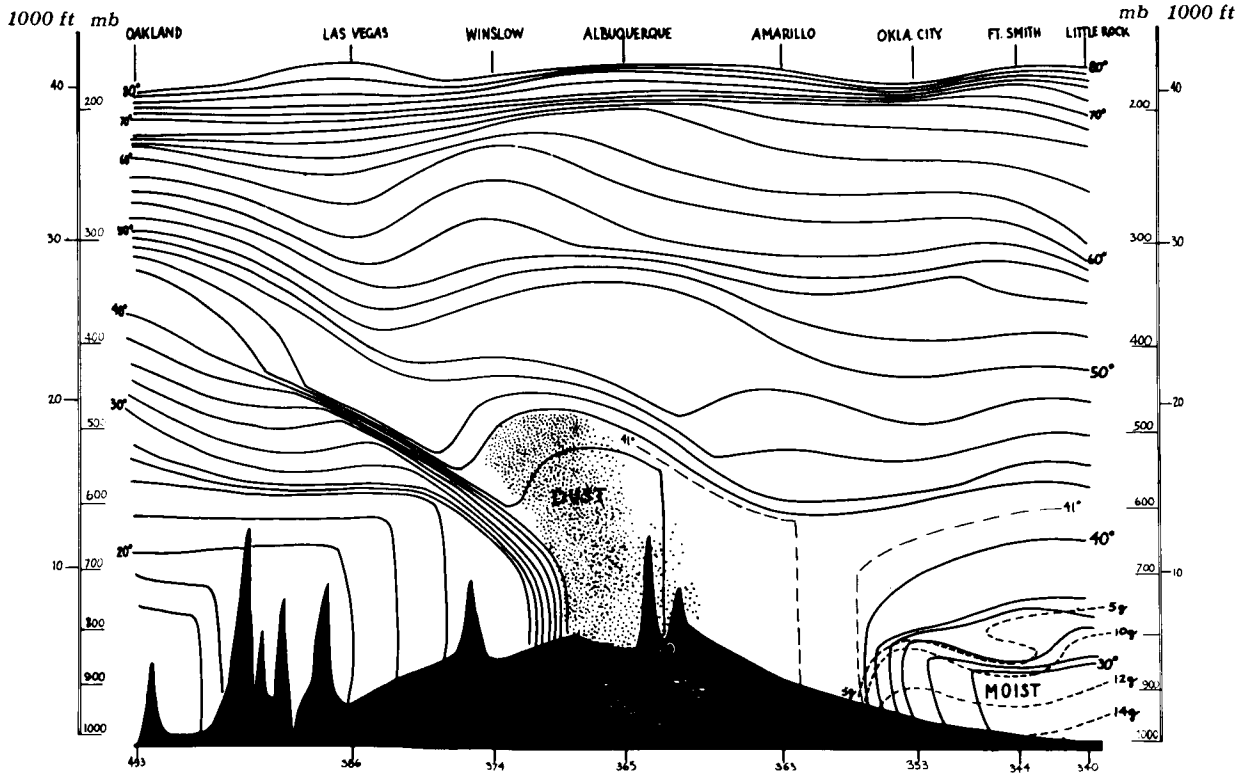


Fig. 8 (*top*). Trajectories computed at θ = 30 C and 50 C. Wind speeds in meters per second are plotted adjacent to each trajectory.

Fig. 9 (*bottom*). Vertical cross section for April 22, 1963 showing vertical extent of dust layer produced by vigorous mixing of high momentum to the earth's surface.



Discussion

Belmont — Is there any difference in magnitudes of these vertical motions say, from those moving from the north and those moving from the south?

Danielsen — The magnitudes I calculate from the isentropic trajectories in the north-to-south motion, which is the descending branch, are frequently of the order of 12, 15 cm/second. These compare to 30, 40, and 50 m/second for the horizontal motion. The vertical velocities are small, but they are significant because the air is a thin film.

The trajectories calculated in the ascending branch are much more complicated. They are often associated with the release of heat. Condensation processes are going on, so one must add a diabatic term to the calculation. I've been plagued with difficulties where the vertical mixing processes are active, so I can't state reliably the values of the ascending motion. They may range between 15 cm/second for the average flow to large values in ascending cumulus cells.

Mantis — I hate to interject a parochial discussion into this biological conference, but I think your major point is well taken and correct that the cyclone-scale motion pumps the stratospheric air out of the stratosphere. Wouldn't you be willing to say, however, that that is a major point and that some of the rest isn't a necessary part of the whole argument? The descending motion doesn't have to look exactly the way your model shows it. Meteorologists all know that we've never found a frontal situation like those one sees in the textbooks, so I'm suggesting that we should concentrate upon this major point and not upon the details of the process.

Danielsen — I did not mention that there is a meteorological parameter one can use to identify the stratospheric air after it leaves the stratosphere. It's called the potential vorticity. I didn't go into it because it is complicated; but let's grant that there is such a parameter. If one measures the potential vorticity and radioactivity one finds that the potential vorticity correlates positively with the radioactivity. This correlation indicates that the stratospheric air comes down from the cyclonic side, that is, the north side of the jet stream, and fans out in the manner I discussed. There is also good evidence now that the downward transport of high potential vorticity is important in cyclogenetic phenomena. I'm not so willing to dismiss it. I think it is an important aspect not only of the cyclogenetic process, but also of the large-scale transport of momentum, radioactivity, and ozone down to lower elevations.

I directed another project in November 1963 at the U.S. Weather Bureau's Numerical Prediction Center. A. Gustafson, one of their research meteorologists, has developed a machine program for computing and analyzing isentropic charts for the whole northern hemisphere. He agreed to calculate similar isentropic charts for me during the project. I chose three isentropic levels each separated by 10° : 290, 300, and 310 Celsius. The potential vorticity was calculated on the intermediate, 300°, surface. Some interesting things occurred. When I traced the air motions on these charts and made prediction of cyclogenesis on this basis, I found that in the two places where strong cyclonic storms developed, over the eastern part of the United States, one could see the downward transport of momentum and potential vorticity.

Neither of these storms was predicted by the current two- and three-level prediction models. In a discussion with Dr. Cressman, the director of the Numerical Center, I pointed out that the lack of vertical resolution in the momentum was responsible for the prediction failure. He then recalculated one case using his new four-level research prediction model, which has a datum level at 300 mb where the high momentum occurred. This model predicted cyclogenesis.

Phillips — I am in an entirely different profession, so I want to make sure that I understood you. You gave a good example of the way by which a certain amount of tropospheric air can be brought into the stratosphere. The interest in the troposhere is that only here can you have any biological materials present in any great amount. One must obviously, of course, have an equal amount of air returned or else he runs out of stratosphere. You brought up examples of dust over a storm area that would get up about to the tropopause. Do you think there is much transport through the boundary, and once aerosol particles get into the stratosphere, will they be mixed and well distributed in the stratosphere?

Danielsen — We're trying to study this now. As I pointed out, however, the ascending branch of the flow is more difficult to study because one must consider diabatic processes. I have worked on two cases that indicate an intrusion process, that is, where tropospheric air is drawn into the stratosphere. When the tropospheric air enters the stratosphere it usually undergoes strong vertical stretching and destabilization. This is the potential vorticity concept. As the air enters the stratosphere its vorticity becomes more cyclonic and its stability decreases by vertical stretching. The reduction in stability reduces the barrier to vertical mixing. One might expect, therefore, that the process of intrusion would lead to vertical mixing and the diffusion of tropospheric pollutants in the stratosphere. Moisture could enter the stratosphere by the same process.

One other observation is quite important (concerning the mass budget of the stratosphere). To date, of the thousands of measurements of radioactivity I've looked at, I have yet to find a measurement of high potential vorticity in the stratosphere associated with low radioactivity. This must mean that the high values of potential vorticity originate at rather high elevations on the cyclonic or north side of the jet. There also must be a systematic downward diabatic transport north of the jet. It also implies that the air entering the stratosphere enters at higher values of theta, higher potential temperatures. Then it either mixes diabatically or cools,

and leaves the stratosphere at lower values of potential temperature. I suspect that the thunderstorms in the tropical convergence zone play a rather important role in this exchange because they have the greatest potential for pumping tropospheric air to the highest values of theta, where the air can enter the stratosphere, move northward, and sink. This route follows that taken by the tungsten 185, which was deposited at about 12° north latitude.

Another factor one must recognize is the seasonal variation in the amount of air in the stratosphere. It increases during the fall and winter, decreases during the spring, so there must be a difference in the net inflow and outflow by seasons.

Cole — I have two questions. Number one is a confirmation that you said you have observed this during every cyclogenesis. Number two is, have you done anything on a meso or micro scale of a similar nature?

Danielsen — Perhaps I should qualify by saying that every cyclogenetic case I have studied indicates this. There may well be cyclogenetic cases that do not have a stratospheric outflow. At present we are working with a numerical prediction model, consisting of 18 isentropic levels. We are trying to set up proper initial conditions to see if the tendency for tropopause folding is real. I think the tendency for folding is characteristic of the whole atmosphere not just the tropopause.

No, (second question) I haven't analyzed the motions on a smaller scale because it goes back to the pertinent point Dr. Wiin-Nielsen raised, that you're defeated; you don't have the data. Recently we ran a special project in the southeastern part of the United States to obtain more accurate and frequent data. Radiosondes were released every hour-and-a-half at three stations and every three hours at a grid of Weather Bureau stations. We also tracked some of the radiosonde balloons with the FPS-16 precision radar. We haven't received the data yet but this data should permit analysis of a smaller scale.

Atomic Energy Commission High Altitude Sampling Systems

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Abstract

23984

The Fallout Studies Branch of the Division of Biology and Medicine, Atomic Energy Commission is actively engaged in the research and development of stratospheric sampling systems for the collection of radioactive particulate and gaseous contaminants. Operational balloon sampling sites are maintained at Goodfellow Air Force Base, San Angelo, Texas, and Mildura, Australia. The sampler systems under development fall into two categories: 1) balloon borne from 65,000 to 130,000 ft altitudes, 2) rocket borne from 130,000 to 250,000 ft altitudes. The balloon-borne samplers under development employ either filter paper, impactors, or electrostatic precipitators as the collection mechanism. The rocket-borne samplers will employ either large area filtration, cryogenic freeze-out, or cryogenic sorption as the collection mechanism. The operational requirements of altitude capability, sample size, and sampler system recovery and the design parameters of weight, sampling rate, vehicle, and sampling mechanism are described.

The high altitude sampler development program of the Fallout Studies Branch is an integral part of the atmospheric radioactivity and fallout research program of the Division of Biology and Medicine, U.S. Atomic Energy Commission (AEC) (1). The basic aims of this program have been 1) to determine the actual activities of atmospheric radioactivity at various altitudes of the upper atmosphere, 2) to develop improved samplers and techniques, both balloon-borne and rocket-borne, 3) to improve sampling efficiency and reliability, and 4) to extend the sampling capability to higher altitudes. The high yield and high altitude nuclear test explosions of 1958 to 1960 and 1961 to 1962 injected considerable amounts of radioactive debris into the upper stratosphere and mesosphere, creating the need for extending and developing the sampling capabilities to higher altitudes than were currently being reached by balloons (6).

In the future it is reasonable to expect that even with an effective weapons test ban treaty, the continued development of peaceful uses of nuclear energy, particularly in space, could contribute measurably to the existing levels of atmospheric radioactivity. Programs for the development of nuclear reactor powered rockets (ROVER program), and the utilization of systems for nuclear auxiliary power (SNAP) devices for electrical power sources are under way. The potential hazards from releases of relatively long-lived isotopes (Sr^{90} in some SNAP devices) due to burnup and disintegration during re-entry, from reactor-powered rocket effluents, and from releases due to planned or accidental destruction (aborts) must be considered and carefully evaluated.

The Atomic Energy Commission balloon sampling program was initiated in 1956 to obtain stratospheric particulate samples for radioactivity measurements. From these samples, the activities and distributions of the radionuclides of interest (Table I) are determined. These data in conjunction with similar data from Department of Defense high altitude sampling programs (HASP and Stardust) (7), AEC ground level precipitation and air samples, and with meteorological support and interpretation by the U.S. Weather Bureau are used to estimate the radioactive debris residence times and inventories at the various altitudes, to further the understanding of atmospheric transport, circulation and mixing, and to predict the deposition rates and world-wide distribution of the radioactive fallout when it ultimately reaches man's environment.

In 1956 balloon sampling sites were established with the cooperation of the U.S. Air Force at two United States locations: in the Panama Canal Zone and in Brazil. In 1959 these were reduced to one U.S. site, San Angelo, Texas (31°N), operated by the U.S. Air Force; a southern hemisphere site at Mildura, Australia (34°S), operated by the Australian government was established. Monthly sampling flights at 65,000, 80,000, 90,000, and 105,000 ft are currently being made at these two locations.

Table I

Isotopes Analyzed on Filter Samples*

Radionuclide	Source & Interest
Mn^{54}	USSR fall 1961 series
Fe^{55}	USSR fall 1961 series
Sr^{89}	Fresh fission debris
Sr^{90}	Long-lived fission debris
Zr^{95}	Fresh fission debris
Rh^{102}	U. S. high altitude Orange shot, Aug. 1963
Sb^{124}	USSR fall 1961 series
Cs^{137}	Long-lived fission debris
Ce^{144}	Long-lived fission debris
Cd^{109}	U. S. high altitude Starfish shot, July 1962
Ba^{140}	Fresh fission debris
Ce^{141}	Fresh fission debris
Pm^{147}	Long-lived fission debris
Pb^{210}	Naturally occurring isotope
Po^{210}	Naturally occurring isotope
Be^{7}	Cosmic ray induced isotope
P^{32}	Cosmic ray induced isotope

*Health and Safety Laboratory-138 Fallout Program Quarterly Summary Report, July 1, 1963.

The first operational high altitude sampler, nicknamed "Ash Can," was developed by General Mills, Inc. in 1956 (Fig. 1). The Ash Can sampler used a centrifugal blower to pull the air through 5 ft^2 of cylindrically mounted filter paper (IPC 1478) at face velocities of approximately 100 fpm. The physical properties of IPC 1478 filter paper are shown in Table II.

At this face velocity the filter media were only 25 to 50% efficient for the retention of submicron particles; the samples obtained showed poor reproducibility.

Further laboratory investigation (8, 2) showed that the efficiency of IPC 1478 filter media for the retention of submicron particles increased with increasing face velocities and decreasing absolute pressure. (Maximum retention was at face velocities of > 500 fpm.) Other filter media with high retention for submicron aerosols have been examined, but all are relatively less useful for high altitude air sampling applications due to their high pressure drop-flow relationships that would require the use of large unwieldy areas of the filter media to obtain the same volume of flow.

Because of its low pressure drop and relatively high efficiency at high flow rates, IPC filter media were used by General Mills, Inc., in the initial development of the direct flow sampler system in 1957 (9). This sampler used 1 ft^2 of IPC filter media mounted normal to the air flow produced by a centrifugal blower (Torrington 704) driven by a 1/2 hp dc motor (Westinghouse) (Fig. 2). With this air mover and the reduced area of filter paper, face velocities above 500 fpm and increased collection efficiencies were obtained up to altitudes of 90,000 to 100,000 ft. The direct flow system has been continually improved since its inception, by the removal of the 40-ft polyethylene exhaust ducts that contributed to motor burnout by twisting and kinking, improved doors, latches and dust seals, and a reduction in weight through the use of aluminum spinnings and improved assembly methods. The latest model, known as DFS-2B (Fig. 3), is currently operational at both balloon sampling stations and has extended the sampling altitude to 105,000 to 110,000 ft. In Table III are shown typical volumes and ambient sampling rates at the various sampling altitudes.

Concurrent with the sampler development has been the development of altitude sensors and flowmeters. The reduction and interpretation of the sample data require an accurate measure of the ambient volume sampled and the ambient pressure. Initially the ambient sample volume was determined from telemetered fan rpm data using fan rpm vs. flow rate at applied voltage relationships supplied by the manufacturer. This proved unsatisfactory due to telemetry failures, motor burnout, erratic rpm data, lack of control of the applied voltage, and unreliability of the voltage-rpm-volume flow relationships as the sampling altitude was increased from 90,000 to ca. 105,000 ft. This led to the development by General Mills, Inc., of the PR-2 flowmeter, a low drag, propeller type anemometer having an output that is a linear function of the volume flow. Each 100 revolutions of the propeller is registered on the electromechanical counter (Veeder-Root).

Table II

Physical Properties of Institute of Paper Chemistry IPC Filter Media*

Material	Cellulose
Mat thickness	0. 084 cm
Avg. fiber diameter	17 μ
Fiber density	1. 49 g/cm^3
Bulk density	0. 189 g/cm^3
Avg. pore diameter	170 ± 60 μ
Porosity	90%
Fiber volume	10%

*Filter media is impregnated with Kronisol, an organic adhesive, to improve particle retention. Kronisol is di-butoxyethyl phthalate.

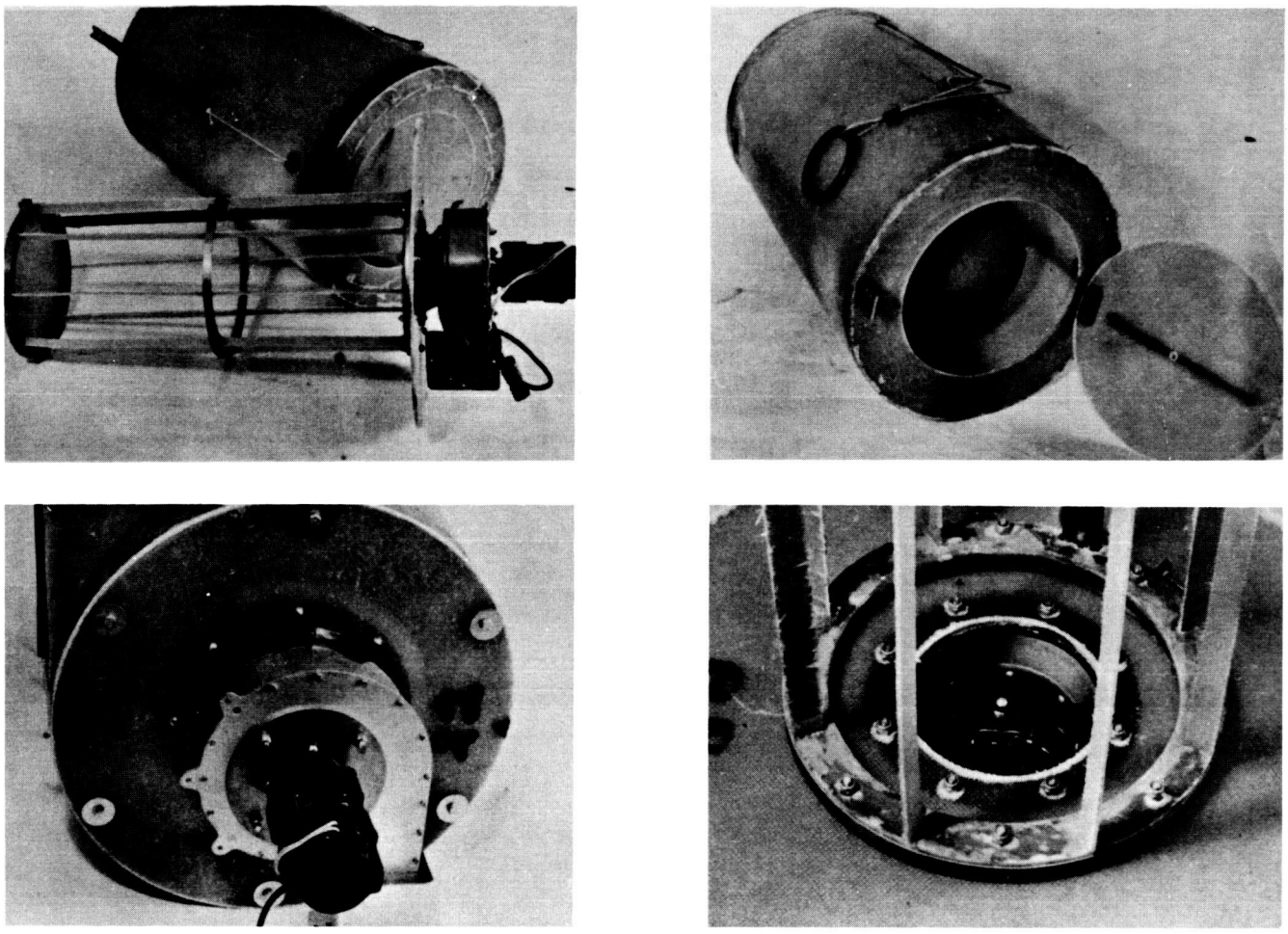

Fig. 1. Ash Can balloon-borne filter system. *(Upper left)*, filter frame and housing. *(Upper right)*, entrance door open showing head of filter frame. *(Lower left)*, blower end showing blower and motor. *(Lower right)*, filter frame showing detail of magnetic tachometer.

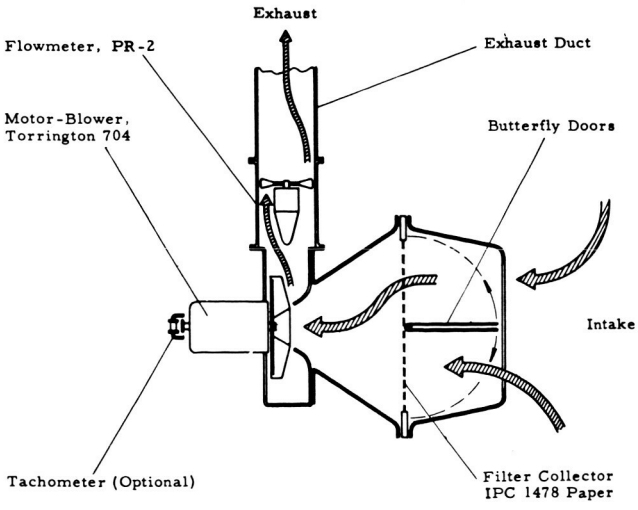

Fig. 2. Direct flow sampler, schematic.

The early Ash Can samplers used smoked disc hypsometers and barographs to record altitude. These were replaced by the barocoder, an aneroid device with telemetry.

The B-58 high altitude barotransmitter, developed by General Mills, Inc. to replace the barocoder, uses an aneroid pressure altitude sensor from 0 to 90,000 ft having an accuracy of ±500 ft and an ionization type digital pressure transducer from 90,000 to 140,000 ft having an accuracy of ±1,000 ft and a resolution of 500 ft. The signal is telemetered and the unit can serve in a dual capacity as a radio direction finder source. The B-58 barotransmitter is currently in use on sampling operations at San Angelo, Texas.

Experience with the direct flow system (DFS) indicated that the altitude region of 100,000 to 110,000 ft was probably the ceiling for efficient, reliable operation primarily due to payload weights and blower limitations. To extend the direct flow sampler concept to altitudes above 110,000 ft would require either a reduction in the overall system

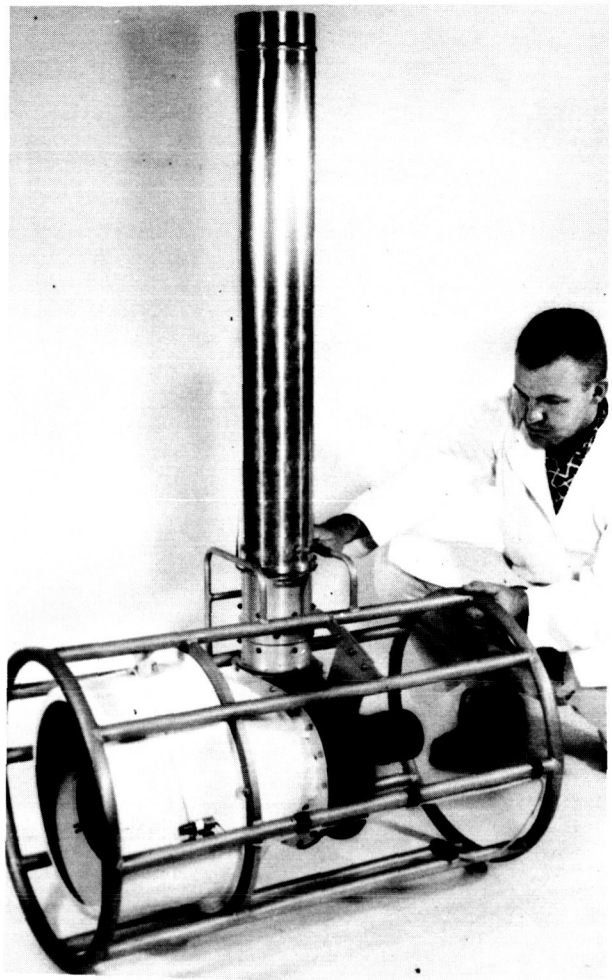

Fig. 3. Direct flow sampler.

pressure drop or an increase in the air-moving capability. The system pressure drop could be reduced by increasing the filter area or by using lower pressure-drop filter media; however, the problem of heat dissipation of electrically driven fans or blowers becomes more acute as the ambient air density decreases. Also the weight of batteries required for the long sampling periods soon becomes prohibitive.

With the advent of high-altitude nuclear-debris injections, further emphasis was placed on the development of a light weight, high volume, balloon-borne sampling system capable of extending the sampling range to the 120,000 to 150,000 ft region.

During 1961 to 1963 General Mills, Inc. established the feasibility, designed, and built two experimental models of an air ejector powered filter sampler (4) that theoretically would operate up to the ultimate balloon ceiling (Figs. 4, 5).

The mechanism of the air ejector operation is relatively simple. A jet of high velocity primary gas is injected into a constant area mixing tube from a carefully sized nozzle. The high velocity primary gas expands and by a turbulent exchange of momentum creates a partial vacuum causing the secondary or ambient air to flow through the system. Mass augmentation factors of ca. 3 (mass of ambient air/mass of primary air) have been obtained on the recent test flights using pressurized N_2 as the primary gas. Nitrogen gas and compressed air appear to be the most practical of the primary gases investigated.

A two stage, multi-jet, impactor-filter sampler (5), having a first stage cutoff at 0.3 μ and a second stage cutoff of 0.03 μ is being developed for use with the air ejector.

It has been predicted that air ejector powered systems will be able to sample $10^5 ft^3$ of ambient air at ca. 1,000 cfm up to altitudes of 150,000 ft.

The air ejector system will not have the heat dissipation and battery load problems inherent in air-density-dependent electrical blower systems. In addition, the sampling time, rate of sampling, sample volume, and filter media area can be varied to meet specific sampling requirements. In Figure 6 is shown the time required for sampling and the sample volume obtained for the current experimental air ejector powered sampler. Evaluation flight testing of air ejector systems will continue through 1964.

An electrostatic precipitation sampler system is being developed by Del Electronics Corp. The feasibility study Model I sampler has been successfully flight tested at 105,000 ft and 127,000 ft (3). This system was of a single-stage coaxial wire in cylinder configuration (Figs. 7, 8). Sampling flow rate was designed for 100 cfm, but it was found that particle charging was more efficient than theory had predicted, particle deposition was almost simultaneous with charging, and corona current values were conservative indicating that a higher sampling rate could be used. As a result the second (105,000 ft) and third flights (127,000 ft) were at 200 cfm flow rate. Radiochemical data from the two 105,000-ft flights showed excellent agreement with that from the DFS samplers flown alongside. Design criteria for the prototype sampler are to sample 500 scf at 150,000 ft altitude, and maintain a total weight of

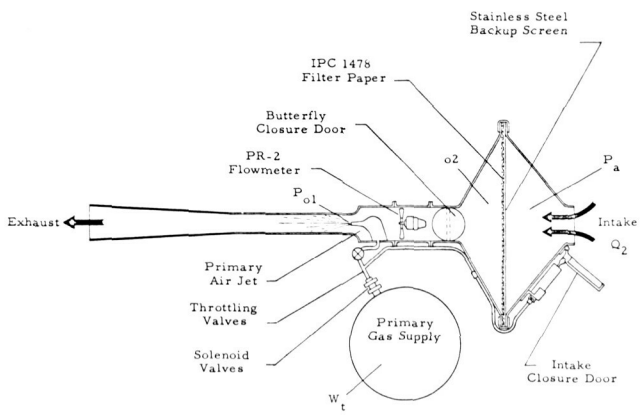

Fig. 4. Air ejector powered filter sampler, schematic.

Table III

Direct Flow Sample Data, October 1963, San Angelo, Texas

Altitude, ft	Sample volume ft^3, STP*	Sampling rate, acfm**	Sampling time, min
65,000	1,905.3	637.5	45
	1,990.3	665.9	
80,000	1,770.9	642.7	83
	1,831.6	664.7	
90,000	1,483.4	557.5	136
	1,494.6	561.8	
105,000	895.4	337.5	240
	823.9	310.5	

*Standard temperature and pressure.
**Ambient cubic feet per minute.
Note: Two sampler systems flown on each flight. Data based on PR-2 flowmeter counts.

less than 70 lb. Design concepts to accomplish this envision a modular configuration using a 2,000 cfm electric powered fan as the air mover. In contrast to the filter and impactor systems, this system can use a fan because the system pressure drop will be low. The sampler will employ clusters of strippable copper-coated mylar cylinders geometrically packed to minimize the overall size. Each cylinder will be a single-stage coaxial wire-in-cylinder precipitation section, approximately 2-1/4 inch diameter by 24 inch long, rated at ca. 50 cfm. The module will be wired in parallel to minimize the loss of sampling in the event any cylinders in the cluster fail. Thus, to sample 500 scf, at 150,000 ft a modular cluster of 37 cylinders sampling at 1,850 cfm will be required. In Table IV is shown sample volume vs. altitude for two flow rates and two configurations.

Del has also developed an altimeter based on the air-density-dependent discharge current between two electrodes. A recording version of this instrument has been successfully test flown and is now being miniaturized and redesigned for telemetry. An ion tracer type flowmeter is also being developed for use with the prototype sampler. It is estimated that this sampler system will be ready for flight testing by mid 1965.

In Table V are summarized both the operational and developmental balloon sampling systems.

IPC Filter media were developed for and have been used extensively in air sampling programs since 1949. Its physical and chemical properties, good retention for submicron particles, and adaptability to radiochemical analysis were drawn on for use in the high altitude samplers.

The electrostatic precipitator will use less than 1 g of pure copper as the deposition surface. After acid solution of the sample and copper substrate, the copper can easily be removed and radiochemical analysis of the sample performed.

Neither IPC filter paper samples nor the electrostatic precipitator samples are useful as taken for microscopic analyses or particle sizing.

Table IV

Model II Electrostatic Precipitator, Sample Volume vs. Altitude

Sampling rate, acfm*	950	1,850
Sample vol. ft^3 scf** at		
110,000 ft	1,400	2,750
130,000 ft	560	1,100
150,000 ft	245	480
Est. sampler weight, lb (for 150,000 ft)	21	33
Est. system weight, lb (for 150,000 ft)	46	58
No. cylinders	19	37

*Ambient cubic feet per minute
**Standard cubic feet, volume at STP of 59 F and 1,013 mb.
Del Electronics Corp. Progress Report, October, November 1963.

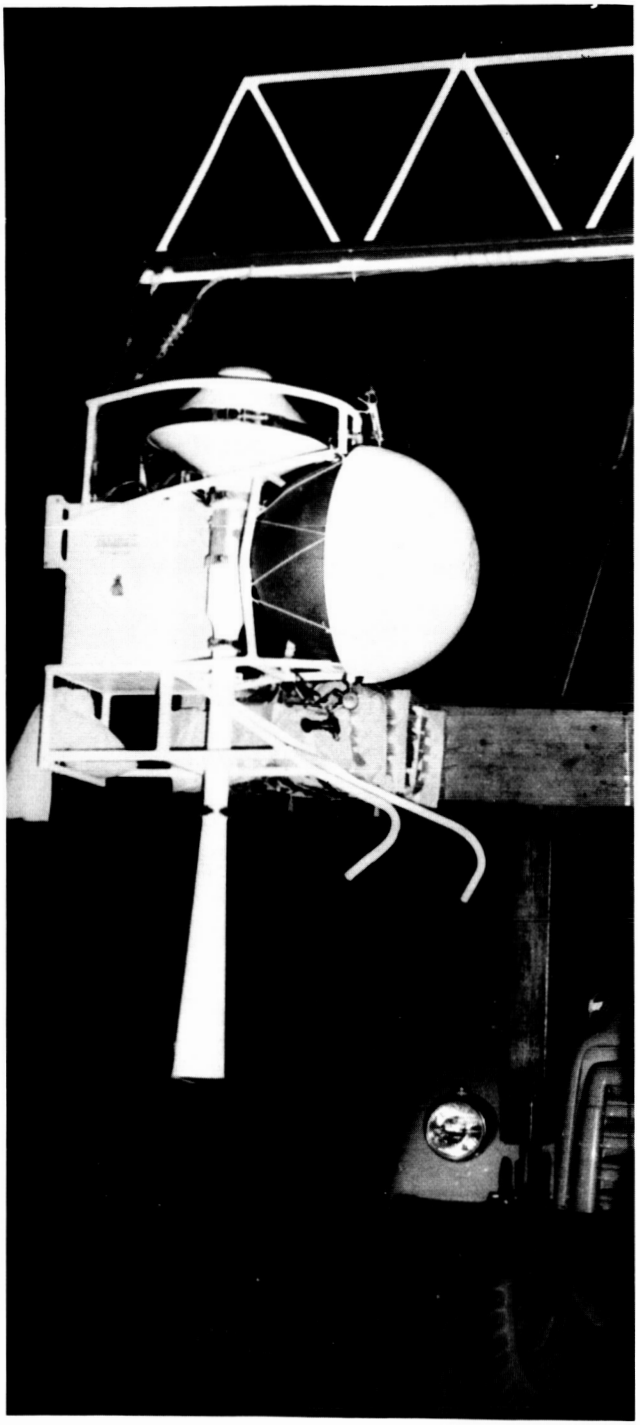

Fig. 5. Air ejector powered filter sampler (One of two mounted on truck in launch condition).

The samples obtained from the multijet impactor will be in a condition amenable to microscopic study and analysis. In addition some of the jets will be designed to permit the use of electron microscope grids, permitting much needed particle sizing studies.

The development of rocket-borne sampling systems was undertaken to acquire a sampling capability above balloon ceiling. This required the development of a system to operate in the 130,000 to 250,000 ft region of the atmosphere. Many sampling concepts were considered ranging from impactors and filters to cryogenic surfaces (6).

The Sand Lo systems developed by the Sandia Corp. employ large areas of pleated IPC filter paper supported on two extendable blades (Fig. 9). The sampler is separated from the second stage of the Honest John-Nike rocket system at apogee, then is retarded and stabilized by a large specially designed parachute. After retardation and stabilization the case opens and two 7-ft sampling blades extend; these contain 84 ft ^{2} of IPC 1478 filter paper, and are rotated at 5 to 10 rps by hydrogen peroxide jets. At the end of the sampling period the blades are retracted, the case sealed, and the system is lowered to earth by parachute, and is recovered. This system is designed to sample only particulate matter from near the rocket apogee, 200,000 to 250,000 ft, to 130,000 ft. The volume of air sampled will be based on laboratory calibration of the flow characteristics of the sampling surfaces. Development of new or improved low-pressure-drop filter media is being conducted. The first flight test was only partially successful since a dynamic instability developed which sheared off the sampling blades shortly after the sampling period began.

In cooperation with the Air Force a second rocket sampling system is being developed. This system, known as air launched air recovered rocket (ALARR), will use a cryogenic sorbtion pump, developed by Varian Associates, to sample the whole air. The modified rocket nose cone will contain a liquid nitrogen cooled activated charcoal adsorbtion bed to sample the gaseous portion of the atmosphere, and activated carbon felt to filter the particulate. This system will be launched from an aircraft in flight, sample as programmed, and be recovered during parachute descent by aerial recovery. Sampling missions will be either vertical, sampling a column of air through the altitude range during parachute descent, or incremental by launching at a lesser angle of attack and sampling a relatively narrow altitude band during ballistic flight. Sample volumes (Table VI) are dependent upon the type of mission, the altitude, and the launch conditions. This system is undergoing flight tests at the present time.

The Air Force Cambridge Research Laboratory has developed, through a contract with the Aerolab Development Corp. a cryogenic whole air sampler (CWAS) (Fig. 10). This system (carried on an Aerobee rocket) is designed to sample isokinetically on ascent from 130,000 ft to apogee, 250,000 to 300,000 ft by cryogenic freezeout of both particulates and gaseous components of the air on a liquid hydrogen cooled heat exchanger. The sampler is designed so that the cryopumping action will be great enough to continuously swallow the shockwave, therefore, there should be no spillage or fractionation of the sample. During a maximum trajectory of 130,000 to

Table V

Balloon Sampling Systems

	DFS*	Air ejector	Electrostatic precipitator
Vehicle	Balloon	Balloon	Balloon
Sampling mechanism	Filtration	Filtration, impactors	Electrostatic precipitation
Type of sample	Particulates	Particulates	Particulates
Volume of sample	500-2,000 scf**	1,000 scf	500 scf
Sampling altitude	65,000-110,000 ft	65,000-150,000 ft	65,000-150,000 ft
Sampler payload weight (2 samplers, including batteries & instruments)	350-500 lb	300-400 lb	120 lb

*Direct flow sampler
**Standard cubic feet

300,000 ft about 0.5 kg (17 scf) of air should be sampled. This system will require fixed launch sites and ground recovery after parachute descent; the system however, could be adapted for aerial recovery.

A comparison of the three rocket sampler systems described is shown in Table VII.

The major aim of the rocket-borne sampler program has been to develop a system which will obtain a representative portion of the atmosphere that is sampled. This requires that the sample be unfractionated and that the volume of the sample be accurately known. In the case of whole air samplers the volume can be measured directly by pressure-volume-temperature relations and indirectly from known atmospheric constituent concentrations or ratios. Sample volume measurements for filter devices such as Sand Lo will depend upon laboratory measure of the flow characteristics of the filter media and the aerodynamics of the device. This will be a complicated task and probably will result in only a semi-quantitative volume measure. The efficiencies of the whole air samplers should approach 100% if sampling is isokinetic as determined by volume measurements and constituent ratios. The efficiency of filter samplers will again be dependent upon laboratory data based on the particulate sizes and flow velocities expected. The sampling volume of cryogenic systems is limited in that cryo-pumping will stop when the internal temperature rises to the point where the internal pressure, due to noncondensables and the entering gases not being condensed and chilled at a fast enough rate, equals the ambient pressure. Filter samplers, such as the Sand Lo have the capability of sampling large volumes of air and are dependent upon descent rate, rotational speed, and filter area. Whole air samplers in addition to furnishing particulate and atmospheric composition data should be useful for studies of the trace gas constituents of the atmosphere.

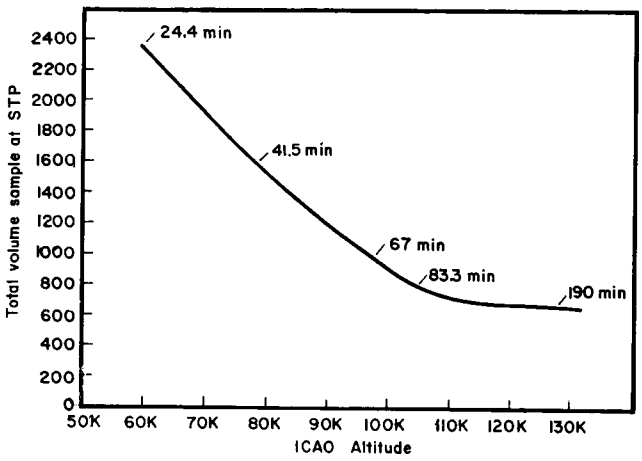

Fig. 6. Performance characteristics of experimental air ejector powered sampler. Standard temperature and pressure (STP) volume capabilities of air ejector filter sampler at 60,000 through 130,000 ft. Includes required sampling times. Filter area = 2.0 ft^2. Ambient sampling rate = 1,000 cfm. Primary mass = 40 lb.
International Civil Aviation Organization (ICAO).

Conclusions

In conclusion it is expected that the eventual operational use of these systems will extend the sampling capabilities to higher altitudes and permit operations at arctic and tropic sites. The samples will yield invaluable data on atmospheric radioactivity, both natural and man-made, atmospheric composition, and experimental support for meterological models of atmospheric transport, mixing, and circulation applicable not only to fallout, but to other problems of interest to the general scientific community.

Table VI

Sampler Volume vs. Altitude, ALARR* System with Varian Sampler

Altitude, ft	Volume, scf**	Volume, ambient ft^3
	Subsonic launch	
250,000-130,000	6	13,873
200,000-130,000	6	7,074
150,000-130,000	1	137
	Supersonic launch	
250,000 (29,000-ft band)	0.1 x 10*** = 1	
200,000 (33,000-ft band)	1.0 x 10 = 10	
150,000 (20,000-ft band)	5.0 x 10 = 50	

*Air launched air recovered rocket.
**Standard cubic feet.
***It is estimated that the ram effect at supersonic velocities may increase sample size by as much as a factor of 10.

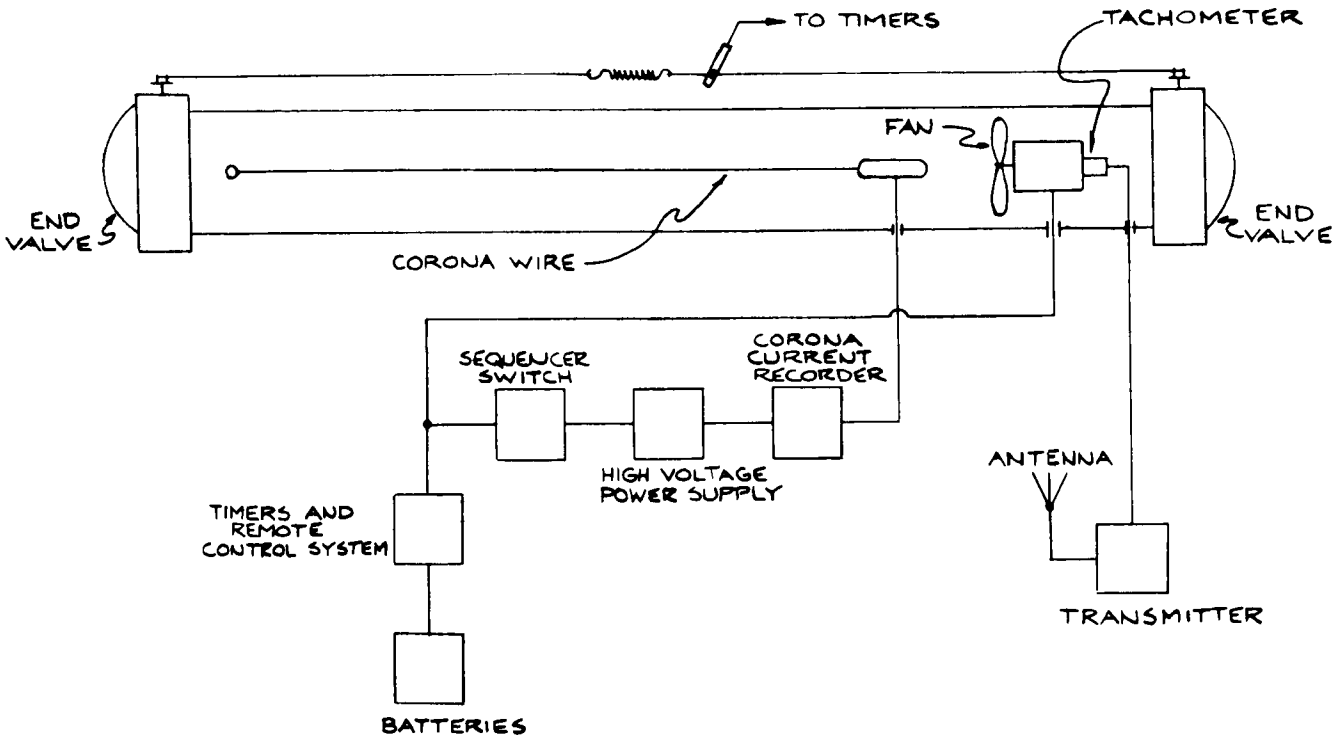

Fig. 7. Model I electrostatic precipitator, schematic.

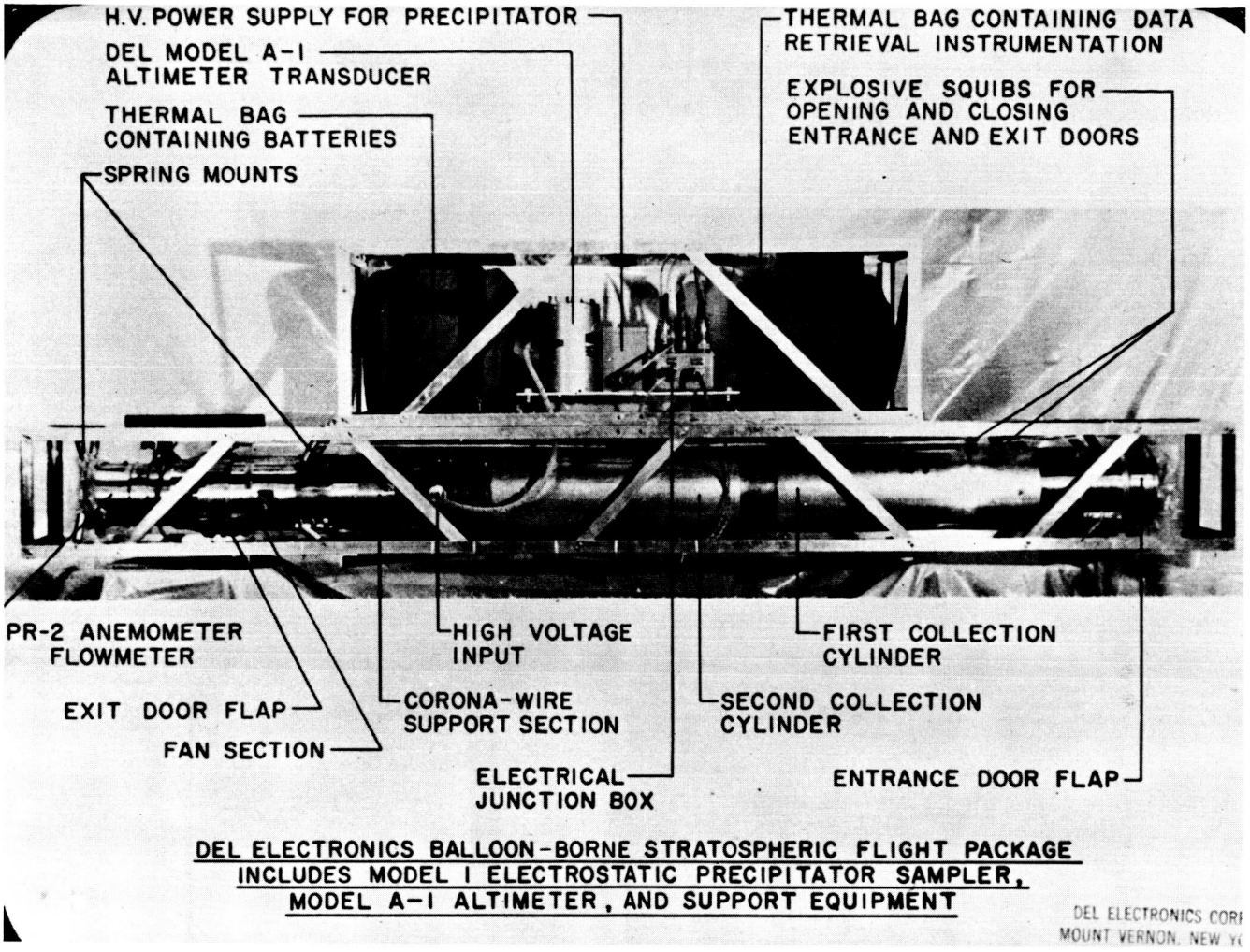

Fig. 8. Model I electrostatic precipitator.

Table VII

Summary of Rocket Sampler Systems

	Sand Lo	ALARR*	CWAS**
Vehicle	Honest John-Nike	Genie	Aerobee
Altitude x 10^3 ft	130-200	130-250	130-300
Volume of sample, scf***	10^2-10^3	6	17-35
Type of sample	Particulates	Whole air	Whole air
Sampling mechanism	Filtration on IPC filter media	Adsorbtion & filtration	Cryogenic freezeout
Weight, lb	700-800	115-140	448

*Air launched air recovered rocket.
**Cryogenic whole air sampler.
***Estimated and dependent on flight trajectory.

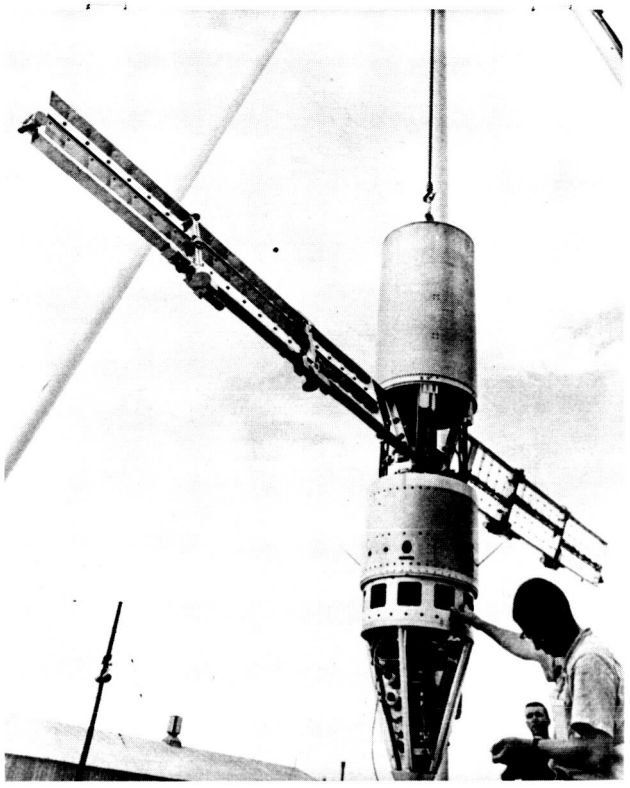

Fig. 9. Sand Lo rocket-borne sampler.

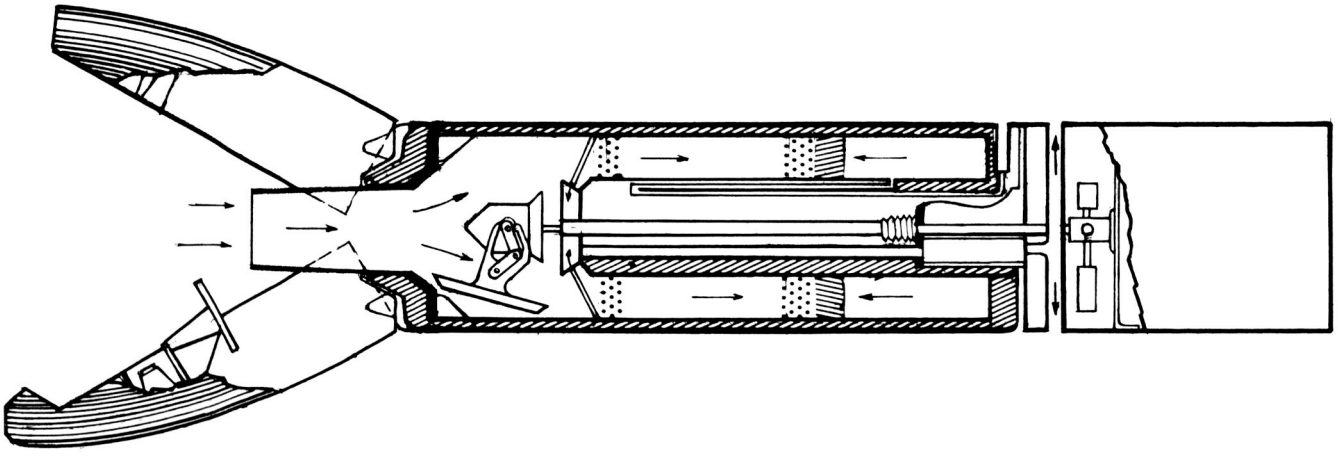

Fig. 10. Cryogenic whole air sampler.

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Discussion

Greene — Can you tell us approximately what does one of these aerial recoveries cost?

Beadle — Aerial recovery, I do not know. I can tell you this much, that a C-130 aircraft, when available, will run something like $500 an hour. That is a small part of the item.

Greene — If you are going to launch from an island in the Pacific, as you say, you must obviously have more than one aircraft in the air. I would like to get a general idea of how many you need at a time.

Beadle — This program is not firmed up yet to the point where we have figures and know just what our flight program will be. The aerial recovery is in a feasibility study stage as far as we are concerned. We just recently heard that Air Force scientists are practicing with some of the currently used gondolas (payloads).

Phillips — The IPC paper that you were talking about, is that from Institute of Paper Chemistry? Is it a cellulosic fiber?

Comment from audience — Yes. That's IPC 1478 paper.

Phillips — You never use all-glass filters?

Comment from audience — No. I believe Air Force Cambridge Research Labs has used the polystyrene Delbag type. These all have too much pressure drop to be useful at these altitudes.

Beadle — We just use pure cellulose paper. It is a special cut, special manufacture.

Greene — One comment to Dr. Phillips: The paper contains a germicide. As it comes from the factory there is a bacteriostatic agent incorporated into the fibers of IPC paper, so it won't do you much good for subsequent viable enumeration.

Oswald — On your electrostatic precipitator work, you gave some data which I couldn't hear. Would you please repeat the data that was given on the electrostatic precipitator? You said you had one flight that collected...

Beadle — This has been flown to 105,000 ft. I presume this is the type of data you want. It collected at 100 cfm flow rate about 167 scf of air. This was a feasibility study model. The proposed operational model will sample 500 scf at 150,000-ft altitude at about 1,850 cfm flow rate. It will be a 37-tube package, smaller tubes.

Oswald — On this one trial flight, did you make any evaluations of the particles that were collected?

Beadle — On all these flights, radiochemical analysis was made. This compared favorably with direct-flow samplers flown alongside as the picture showed. The first flights of this sampler did contain electronmicroscope grids. There was some data obtained from this; you are probably interested in the particle sizes.

Oswald — Yes.

Beadle — So are we. This is crude data. It looks like the sizes fall into an average of 0.01 to 0.1μ. I have no figures on the distribution curve or anything like that.

Oswald — What was the voltage?

Beadle — I believe that in the feasibility study model it operates around 0.06 m amp current-density. This can be upped; charging was more efficient than they thought. Because deposition was almost simultaneous with charging Del Electronics Corp. is going to operate future models at a higher current-density; they can operate at a higher flow then, too.

Dimmick — Do you know whether or not this electrostatic sampler generates any ozone at these altitudes?

Beadle — I don't know. I hadn't thought about that.

Ranz — What analysis of the sample do you plan to make seriously?

Beadle — We are sampling at four altitudes each month at the two sampling sites, San Angelo and Mildura. These samples are on IPC filter paper. This paper is shipped as soon as possible to the Health and Safety Laboratories in New York City; it is either analyzed by this laboratory or by contractors. First, the analysts do a gamma scan for the gross gamma count of radioactivity, and then they do a gamma spectra to ascertain if the sampling is typical of a preceding sample. Then the sample is analyzed for various isotopes by wet chemistry methods. These are listed in Table I of my talk.

Mechanical Methods for Collecting Stratospheric Biological Aerosols

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Abstract

23985

Characteristics of impaction and filtration aerosol collection mechanisms are reviewed with particular reference to high altitude environments. Both theoretical and experimental aspects are considered. Discussions and data (where available) are presented relative to collection efficiency, viability decay, and amenability of sample to subsequent analysis. Examples of current stratospheric particulate sampling apparatus are given, and their applicability to biological aerosol sampling is examined. From data and information presented, the following conclusions are drawn: very high collection efficiencies on particles of microbiological size are more easily obtained as stratospheric pressure decreases (altitude increases). Impaction and filtration methods are most applicable to stratospheric biological aerosol collection requirements of large sample size and low background contamination levels. Rockets, aircraft, and balloon-borne sampling apparatus now exist that could be applied to biological sampling with certain limitations placed upon each. The most serious problem of high velocity aircraft and rocket sampling for viable biological matter is heating the collected particles. Aircraft and balloon-borne samplers are most easily adapted to biological sampling requirements.

Author

1. Introduction

A desirable sampling system for stratospheric biological aerosol collection must necessarily meet the following requirements:

1. Collect a known size sample representative of the space from which it was obtained.

2. Subject the particles being collected to no stress greater than the stress to which the particles have previously been exposed.

3. Preserve the integrity of the collected material from the time of collection to the time of assay.

4. Present the collected material in a state amenable to analysis by a suitable technique.

5. Provide a sample of sufficiently large size, having a relatively low background, to assure a statistically significant signal-to-noise ratio.

In addition to these five attributes, a sampling system must also be both practical and economically justifiable for its considered applications. The above criteria apply to essentially any sampling requirement, but the biological requirement stresses certain of these criteria more than others.

Consider, for example, the problem of quantitative determination of the type and concentration of viable microbiological particles at 20 km altitude. Extrapolating the data of Junge and Manson (13) at 20 km we obtain a concentration of 300 particles/m^3 larger than 0.5 μ diameter. (The 0.5-μ diameter is approximately the lower size limit of bacteria existing as single cells.) Now, air at standard sea level conditions contains on the order of 10^7 to 10^8 particles/m^3 larger than 0.5 μ (26) of which 10^2 to 10^3/m^3 are viable organisms (12). This implies a ratio of total to viable particles of about 10^5 to 1. If all particles in the stratosphere are assumed to be of terrestrial origin, and it is assumed that no decay or decrease in the viable fraction occurs during vertical transport, use of the 10^5 to 1 ratio gives an upper limit for the viable biological concentration of $3 \times 10^{-3}/m^3$. (This is on the order of the biological concentration upper limit determined from the National Aeronautics and Space Administration biological sampling flights.) (9, 10). If the lowest significant number of viable organisms was taken to be 10, a sample size of 3×10^3 m^3 (10^5 ft^3) would be required.

Two important criteria regarding the collection of stratospheric biological aerosols, are that: 1) a large sample must be processed, and 2) the contamination level must be low. Collection techniques that satisfy these requirements are best ascertained by surveying the theoretical characteristics of pertinent collection methods. Impaction, filtration, and electrostatic precipitation are the most suitable. After considering the practical problems, operational limitations and present state-of-the-art knowledge, it appears the mechanical methods — impaction and filtration — are most applicable to stratospheric biological aerosol sampling experiments.

In the following sections it will be shown that with either an impactor or filter system, the sampled volume and the particle collection efficiency increase with sampling velocity, while the area in which the desired particulate material and the undesired contamination is collected remains fixed. This raises the signal-to-noise ratio.

Any of the three basic approaches for transporting the sampling apparatus into the stratosphere — rockets, aircraft, or balloons — could be applied to a biological experiment. Certain limitations must be placed upon each, however. Current aircraft have a service ceiling of approximately 25 km and balloons, 45 km. Above this range, rockets must be used. On the other hand, the application of high-speed rockets (and aircraft) is restricted because of thermal effects.

Consider an aerosol sampler operating from a high performance vehicle. At a distance in front of the sampler the air is macroscopically at rest, although the air molecules are moving rapidly as a result of their thermal energy. If the flight velocity is subsonic, the air is accelerated as the sampler approaches; indeed, boundary layer theory indicates that the molecules at the sampler surface are traveling at flight velocity. In the case of supersonic flight velocities, an abrupt air acceleration takes place across the bow shock of the vehicle. The process of acceleration of air molecules to flight velocity increases their kinetic energy and consequently their temperature. If the process were to take place in a frictionless manner without any heat transfer (isentropically), the air temperature rise from the quiescent state to the state at the aircraft skin, ΔT, would be given by:

$$\Delta T = \frac{V^2}{2 c_p} \qquad \text{I}$$

where V = flight velocity
c_p = constant pressure specific heat of the gas.

A plot of Equation I is given in Figure 1. Note that a temperature rise of about 60 C accompanies the isentropic acceleration of air to sonic velocity.

In actual practice the skin temperature rise is somewhat diminished from the above given values by the effects of friction within the boundary layer. This can be accommodated analytically by the introduction of a recovery factor, r, such that:

$$\Delta T_r = r \frac{V^2}{2 c_p}; \qquad \text{II}$$

r is a function of properties of the air stream and, except at high supersonic Mach numbers (which are not currently of interest in stratospheric sampling situations) is of the order of 0.8 to 0.9.

One implication of Equation II is that the temperature rise is almost independent of altitude, as it may be shown r/c_p is relatively free from density effects. Thus, if the collected sample is directly exposed to the heating effects of a Mach 1.5 to 2.0 air stream, the temperatures encountered will be similar to those found in a standard autoclave, regardless of flight altitude. There are, however, some further aspects that must be considered. At altitudes of 20 km, the ambient temperature may be less than -80 C, in which case a flight Mach number of 1.5 (a flight velocity of 410 m/sec at the ambient temperature) would only bring the sampler skin temperature up to the vicinity of 20 C. Perhaps microorganisms could withstand this stress and still remain viable. A second altitude effect manifests its presence in a difference of heat transfer rates. At higher altitudes, where the air molecules are more sparse, it requires more time to effect the heating. Since large samples and consequently long sampling times are required for stratospheric biological aerosol samplers, however, steady state temperature values would normally govern, and heat transfer rates would be relegated to second order considerations.

The effects of heated air (at sea level pressures) upon the viability of lyophilized *Serratia marcescens* and *Bacillus globigii* have been studied by Greene (11). In his experiments the bacteria were exposed to temperatures of 70 to 200 C for 0.5 to 1.68 seconds. *S. marcescens* showed a log decrease when subjected to 125 C for 1.68 seconds, whereas the hardier *B. globigii* were able to withstand 175 C for the same time period before showing a tenfold viability reduction. Although it is difficult to extrapolate to a stratospheric environment, from the experimental conditions under which these data were obtained, the data do demonstrate that it is desirable to avoid heating the air stream during the sampling process.

II. Theoretical Aspects of Particle Collection

A. Filtration by Fibrous Filters

Collection efficiency of a fibrous filter mat can be determined theoretically by considering several factors:

1. Collection efficiency of an isolated fiber. This is accomplished by assuming the fiber to be an isolated cylinder whose aerosol collection is attributed to the mechanisms of Brownian diffusion, interception, and inertial impaction. Other factors such as sieving, gravity settling, and electrical forces may affect the collection process, but normally do not play a predominant role in stratospheric sampling applications.

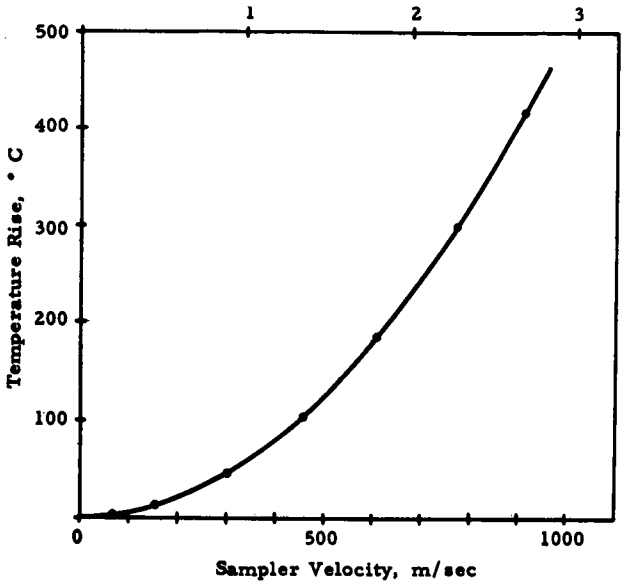

Fig. 1 *(above)* . Temperature rise as result of isentropically accelerating air from zero initial velocity. Sampler Mach number with ambient temperature = 24 C.

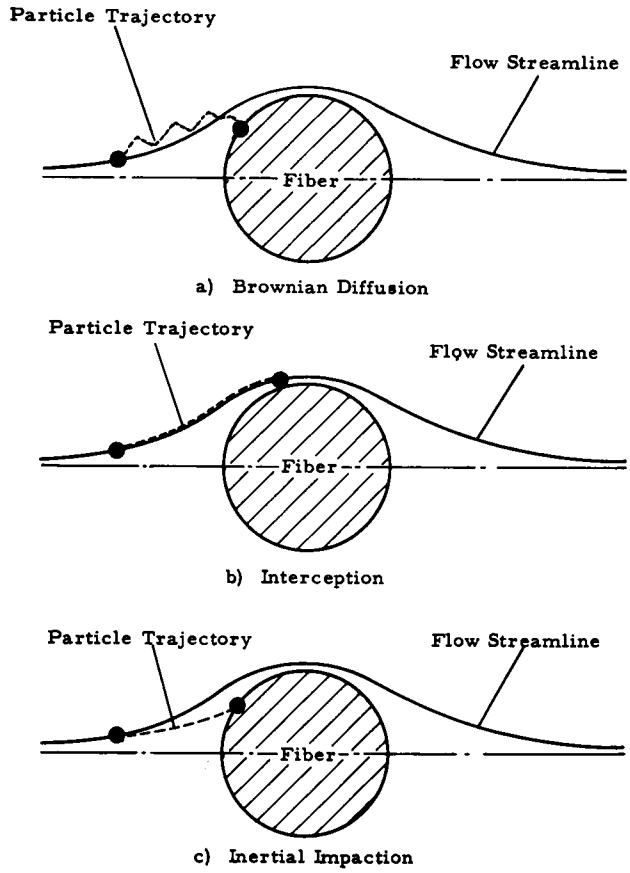

Fig. 2 *(right)* . Particle collection by a single fiber.

2. Change in a single fiber's efficiency when the fiber is subjected to air flow interference from neighboring fibers. This is normally accounted for by a function of the mat-fiber-volume fraction α .

3. Relationship between the collection efficiency of a single fiber and that of the mat.

1. COLLECTION EFFICIENCY OF A SIMPLE FIBER BY BROWNIAN DIFFUSION. Small particles in the air are bombarded by gas molecules and exhibit considerable random Brownian motion, thereby deviating from the streamlines with which they were originally associated (Fig. 2). Some come into contact with the fiber, adhere to it, and are thus removed from the flow system. Particles precipitated in this manner are said to be collected by Brownian diffusion.

The mean square distance $\overline{x^2}$ that a particle travels in a given direction by Brownian diffusion was shown by Einstein (4) to be

$$\overline{x^2} = 2D_{BM}t \qquad \text{III}$$

where

$$D_{BM} = \frac{Ck\,T}{3\pi\mu D_p}$$

C = Cunningham slip correction
k = Boltzmann's constant
T = absolute temperature
μ = air viscosity
D_p = particle diameter
t = time.

The Cunningham correction factor which accounts for departures of the air from continuum theory, when matched with the mean free path length λ given by the International Civil Aviation Organization tables (18), is 17.

$$C = 1 + \frac{2\lambda}{D_p}\left(1.2 + 0.4\,e^{-\frac{0.45\,D_p}{\lambda}}\right). \qquad \text{IV}$$

The length of the mean free path is highly dependent upon altitude, increasing about an order of magnitude each 18 km; it is of the order of one micron at 20 km. Thus, Cunningham's correction is a significant parameter in the consideration of collecting bacterial aerosols from the atmosphere.

Low velocities enhance collection by diffusion because of the increased time factor (Equation III). Particle size is also an extremely important factor. For particle diameters much less than the length of the mean free path, the Cunningham correction is inversely proportional to particle diameter, so D_{BM} is inversely proportional to particle diameter to the second power.

Single fiber efficiency η is defined as the ratio of flow stream area from which all particles are removed to the projected fiber area, both areas taken perpendicular to the direction of free stream flow. Rodebush, et al. (22) computed an approximation to the collection efficiency of single fibers by diffusion mechanism based upon the random walk theory and the assumption of an effective exposure time for the process. Torgeson (30), using a diffusion boundary layer concept, showed that

$$\eta_d = 0.75 \frac{\Psi^{0.4}}{P_e^{0.6}} \qquad V$$

where

$\Psi = \frac{\pi D_f^2}{4 \alpha \mu L} \frac{\Delta P}{V_o}$, the drag parameter

$P_e = \frac{V_o D_f}{D_{BM}}$, the Peclet number

D_f = fiber diameter
α = fiber volume fraction
L = mat thickness
V_o = superficial face velocity of filter mat
ΔP = pressure drop across mat evaluated at particular V_o.

Insofar as the effect of altitude upon the diffusion-collection mechanism is concerned, the value of Ψ for a given filter generally decreases with increasing altitude. But the diffusion coefficient D_{BM} increases with altitude, thereby making the Peclet number smaller. And η_d increases with increasing altitude because $P_e^{0.6}$ (Equation V) decreases more rapidly than $\Psi^{0.4}$.

2. COLLECTION EFFICIENCY OF A SINGLE FIBER BY INTERCEPTION. Particle collection by interception results not from the action of a force but rather from a boundary condition. For pure interception, it is assumed that particles follow the air streamlines around a fiber, and that if a particle happens to be on a streamline that passes within a distance $D_p/2$ of the fiber, it will be collected. Thus, the maximum collection efficiency that can take place by interception is

$$\eta_{i,\,max} = 1 + R \qquad VI$$

where $R = D_p/D_f$, the interception parameter.

Langmuir (22) for viscous flow about the fiber, derived the expression

$$\eta_i = \frac{1}{2(2 - \ln Re_f)} \times \left[2(1 + R) \ln (1 + R) - (1 + R) + \frac{1}{1 + R}\right] \qquad VII$$

where

$Re_f = \frac{\rho_a V D_f}{\mu}$, the fiber's Reynolds number

ρ_a = air density
V = air velocity upstream from the fiber.

This equation can also be modified to account for the combined effects of diffusion and interception.

Torgeson introduced a power-function approximation to obtain (30)

$$\eta_i = 0.0518 \Psi R^{3/2} . \qquad VIII$$

For most high-altitude sampling applications interception is not the governing mechanism because the values of R are small. For commonly used filters composed of cotton fibers, D_f is about 20 μ. Therefore, if the filter is to provide collection of 1-μ particles, R will be about 0.05. For this example, if Ψ were approximately 10, the single-fiber efficiency calculated by Equation VIII would be about one-half percent.

3. IMPACTION. Because of its inertia, a particle does not follow the air streamline exactly when the latter bends around a fiber (Fig. 2). Instead, its momentum tends to make it deviate toward the fiber, an action that may result in the particle's impaction on the fiber, or may bring the particle sufficiently close to the fiber to be collected by the interception or diffusion mechanism.

The motion of a particle in the curvilinear flow field is governed by Newton's second law:

$$m \frac{d\vec{u}}{dt} = \vec{F}_D \qquad IX$$

where

m = mass of the particle
$\vec{u}$ = particle velocity
t = time
$\vec{F}_D$ = drag force.

For the ordinary case of high-altitude particle collection, the Reynolds number associated with the flow of particles across the streamlines is sufficiently small so that the Stokes-Cunningham drag force may be used. This force is determined by

$$\vec{F}_D = - \frac{3\pi\mu D_p}{C} (\vec{u} - \vec{v}) \qquad X$$

where $\vec{v}$ = air velocity.

Thus,

$$m \frac{\vec{du}}{dt} = - \frac{3\pi\mu D_p}{C} (\vec{u} - \vec{v}). \qquad \text{XI}$$

Equation XI can be made dimensionless and expressed in the x and y components as

$$\frac{d^2 x^*}{dt^{*2}} = - \frac{1}{K} \left(\frac{dx^*}{dt^*} - V_x^* \right) \qquad \text{XII}$$

$$\frac{d^2 y^*}{dt^*} = - \frac{1}{K} \left(\frac{dy^*}{dt^*} - V_y^* \right)$$

where

$$x^* = \frac{x}{D_f}, \; y^* = \frac{y}{D_f}$$

$$V_x^* = \frac{V_x}{V_o}, \; V_y^* = \frac{V_y}{V_o}$$

$$t^* = t \frac{V_o}{D_f}$$

$$K = \frac{C \rho_p V_o D_p^2}{18 \mu D_f}, \text{ the inertial parameter.}$$

Since the dimensionless flow velocities for an isolated cylinder are a function only of position and air stream, Reynolds number solution of Equation XII would provide a particle's trajectory as a function of Reynolds number and inertial parameter. The collection efficiency could be determined from analysis of trajectories. But a general solution for viscous flow about the fiber, as is the case in high-altitude application, has not been obtained. Davies (2), using his own set of air-flow equations, made numerical calculations of the collection efficiency of an isolated fiber with a Reynolds number of 0.2. His results include the effects of impaction and interception, and are expressed by

$$\eta_{im} = 0.16 \left[R + (0.5 + 0.8R) K - 0.1052 RK^2 \right]. \qquad \text{XIII}$$

For pure impaction, Davies' collection efficiency would be 0.08 K. Torgeson assumed that the collection efficiency for impaction has the form

$$\eta_{im} = a \Psi K \qquad \text{XIV}$$

and matched the constant, a, with Davies at a Reynolds number of 0.2. Torgeson's combined effects of impaction and interception are expressed

$$\eta_{im} = 0.0518 \Psi \left[R^{3/2} + 0.89 K (0.5 + 0.8R) \right]. \qquad \text{XV}$$

Particle collection by the impaction mechanism is characterized by the impaction parameter K and it is usually the governing mechanism when K is near unity. These values of K are normally associated with large particles, high velocities, and high altitudes such as in the case of filter collectors borne by high-speed aircraft.

4. TOTAL SINGLE-FIBER EFFICIENCY. To combine the effects of the various collection mechanisms, Chen (1) assumed that efficiency by diffusion and interception could be added to that by impaction and interception. The overall collection efficiency, η_o, calculated in this manner is modified to obtain the total single-fiber efficiency η_α (including interfiber interference) by the expression

$$\eta_\alpha = \eta_o (1 + 4.5 \alpha). \qquad \text{XVI}$$

Davies represented the combined effects of the three mechanisms by replacing K in Equation XIII by $(K + 1/P_e)$. To determine the influence of neighboring fibers, he used the analogy of a viscous flow field through a two-dimensional grid and computed the collection by the interception mechanism to be

$$\eta_{i,\alpha} = Rf(\alpha) \qquad \text{XVII}$$

where $f(\alpha) = (0.16 + 10.9 \alpha + 17 \alpha^2)$.

He combined these equations and obtained

$$\eta_\alpha = f(\alpha) \left[R + (0.5 + 0.8 R) \left(K + \frac{1}{P_e} \right) - 0.1052 R \left(K + \frac{1}{P_e} \right)^2 \right]. \qquad \text{XVIII}$$

Torgeson assumed that impaction influences the diffusion process in the sense that the number of particles available for diffusion is increased. He also assumed Davies' correction for interfiber interference and arrived at

$$\eta_\alpha = 0.75 \eta'_{im} + FG \eta_d, \qquad \text{XIX}$$

where η_d is given by Equation V and η'_{im} is related to η_{im} of Equation XV by

$$\eta'_{im} = (1 + 67 \alpha - 107 \alpha^2) \eta_{im}. \qquad \text{XX}$$

The factor G is a function that modifies the diffusion equation for R = 0 to account for interception effects. For small values of R, G = 1. The factor F accounts for the effects of particle inertia on diffusion and is determined by the expression

$$F = 1 + 0.0222 \Psi^{0.6} P_e^{0.6} K \times \left[0.5 + 9.48 \Psi^{-0.4} P_e^{-0.4} \right]. \qquad \text{XXI}$$

5. COLLECTION EFFICIENCY OF A FILTER MAT. Once the single-fiber collection characteristics have been determined, the overall mat efficiency η can be expressed in terms of these and certain mat characteristics. Langmuir (22) has shown this relationship to be

$$\bar{\eta} = 1 - \exp\left[\frac{4\alpha L}{\pi D_f}\,\eta_\alpha\right]. \qquad \text{XXII}$$

6. PROPERTIES OF FILTERS USED IN STRATOSPHERIC SAMPLING SYSTEMS.

a. IPC 1478. This media was developed by the Institute of Paper Chemistry (IPC) (17) in 1949. Historically it has been applied extensively to sampling the stratosphere for radioactive particulates by both balloon- and aircraft-borne systems. It has the advantage of a low background of trace elements. Basically the material is a paper mat formed from second-cut cotton linters and is backed by a cloth scrim for strength. The fibers of the mat are coated with Kronisol, an organic adhesive that prevents bounce-off and re-entrainment of particles collected at high velocities. The Kronisol precludes the use of standard IPC 1478 in biological experiments because it inhibits the growth of microorganisms; untreated IPC paper, however, could conceivably find application in this area.

Stern et al.(28) experimentally determined the collection efficiency of IPC 1478 paper over a range of particle sizes, face velocities, and altitudes. A cross plot of their data, showing the effects of altitude and face velocities upon the retention of 1.2-μ diameter particles is given in Figure 3. For those conditions, the impaction collection mechanism dominates and the retention increases with increasing face velocity and altitude. Since most stratospheric sampling systems operate with face velocities greater than 100 m/minute (Fig. 3) the media will be highly efficient in sampling micron-sized particles.

The energy required to pump air through a filter is reflected by its pressure drop-face velocity characteristics. IPC 1478 is a rather porous material; at an altitude of 30 km, where the total air pressure is 11.9 mb, a pressure drop of 1.19 mb will induce a face velocity of approximately 100 m/minute.

b. Delbag 99/97. This is a commercially available mat manufactured by Delbag Luftfilter GmbH[1] of Germany. It is also known as Microsorban 99/97. The mat, composed of polystyrene fibers with no binder used to hold the fibers together, is fragile. Its collection efficiency for particles in the size range of interest is considered to be 100 % at all face velocities and latitudes. Olson et al. (19) using small test aerosols (88, 35 and 26 mμ), investigated the filter's efficiency over the range of pressures from 30 to 1,000 mb. They could detect no aerosol penetration through the mat and concluded that it was 100 % efficient.

[1]Gesellschast mit beschränkter Hastung

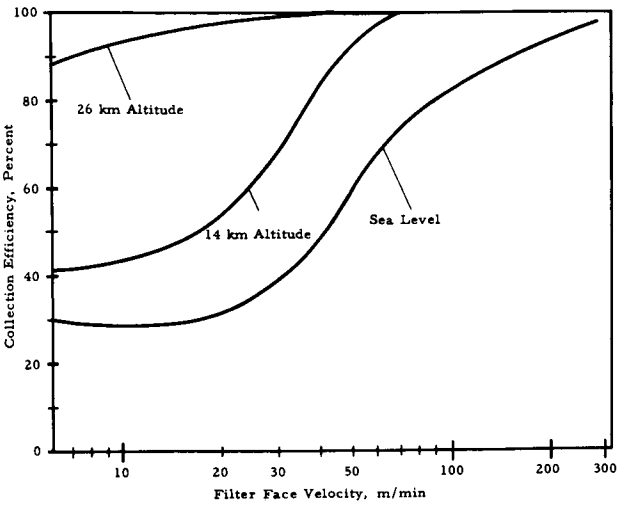

Fig. 3. Experimental collection efficiency of IPC 1478 paper with 1.2-μ diameter test aerosol (adapted from Stern, Zeller, & Schekman, 1960, ref. 27).

The pressure drop for Delbag is about ten times that for IPC 1478 at the same face velocity. To have the same volumetric sampling rate with a fixed pumping or air-moving system, a sampling unit therefore requires an order of magnitude more area for a Delbag filter than for an equivalent IPC 1478.

Microorganisms are difficult to extract from the media, hence its applicability to stratospheric biological sampling is limited.

c. 80-Pore Polyurethane Foam. Polyurethane foam has been used as the collection medium on all flights of the National Aeronautics and Space Administration biological sampler (9). It has the advantages of being chemically and biologically inert; it can be sterilized by autoclaving, and organisms can be extracted from it.

The aerosol retention characteristics of this media are illustrated in Figure 4, which shows the effect of face velocity upon collection efficiency. These data were obtained using a 1-μ bacterial aerosol in a pressure chamber at a simulated altitude of 16 km. As used in the National Aeronautics and Space Administration biological sampling experiment, inertial impaction is the principal mechanism by which this material collects particles. Thus, higher altitudes and larger particle sizes, as well as greater face velocities, enhance the collection efficiency. Polyurethane foam, 80-pore, has good pressure drop characteristics. At 30 km altitude, the pressure drop required to maintain flow across this medium is only about half that for IPC 1478.

Some of the physical properties of IPC 1478, Delbag 99/97, and 80-pore polyurethane foam are compared in Table I. The principal distinguishing property is the fiber size.

Table I

Physical Properties of Stratospheric Sampling Media

	IPC 1478 (28, 32)	Delbag 99/97 (19)	80-Pore Polyurethane foam (32)
Material	Cotton fibers coated with Kronisol	Polystyrene fibers, no binder	Webbed polyurethane fibers, no coating
Mat thickness	0.1 cm	0.2 cm	1.3 cm
Fiber volume fraction	9.6%	7%	3%
Fiber diam, D_f	17 μ	5 μ	37 μ
Weight/unit area	1.5×10^{-2} g/cm^2	1.5×10^{-2} g/cm^2	4.3×10^{-2} g/cm

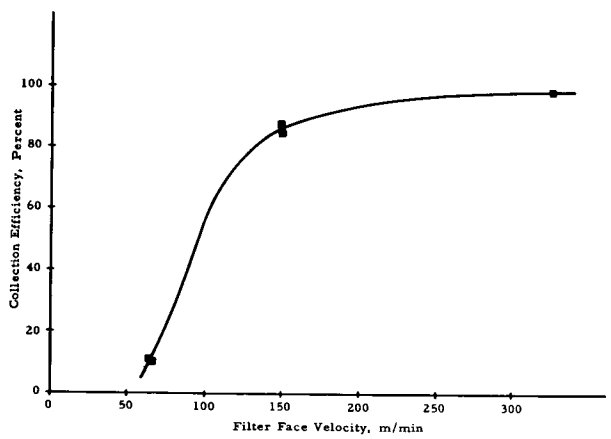

Fig. 4. Experimental collection efficiency of 80-pore polyurethane foam with 1.0-μ diameter test aerosol at altitude of 16 km.

B. Jet Impaction

Consider the typical jet impactor system (Fig. 5). Particle-laden air flows through a circular jet of size D_j. The impactor plate, located a distance L from the jet, causes the air streamlines to bend sharply away from the jet and run parallel to the plate. While the particle's inertia tends to keep it moving toward the plate, the bending streamlines exert a drag force that tends to carry it around the plate. If a particle's inertia is sufficiently large, it will impact on the collection plate.

It is wellknown that the efficiency of an impaction process is characterized by the dimensionless impaction parameter, K, determined by

$$K = \frac{C \rho_p V_j D_p^2}{18 \mu D_j}$$

where C = Cunningham correction
D_j = jet diameter of a circular impactor, or jet width of a rectangular impactor
V_j = air velocity at jet.

But the other dimensionless parameters must also be considered in evaluating the performance of an impaction system. A secondary variable, jet-spacing ratio L/D_j, plays an important part in determining

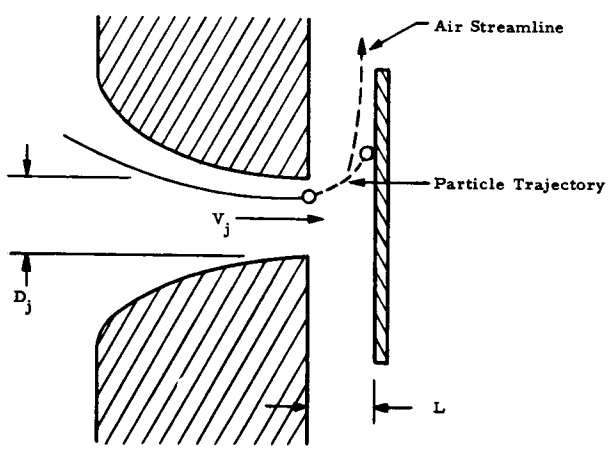

Fig. 5. Jet impactor system. C = Cunningham correction; D_j = jet diameter of circular impactor, or jet width of a rectangular impactor; V_j = air velocity at jet exit plane.

the sharpness of the classification process. Smaller values of L/D_j are normally associated with sharper cutoff characteristics, but if jet spacing becomes sufficiently small, L/D itself becomes a primary variable. This occurs approximately at the point where the jet area equals the area based upon the jet periphery and jet-to-plate spacing. For circular nozzles the value is 0.25 whereas for rectangular jets it is 0.5.

The jet Reynolds number is another secondary variable that can influence the collection process. At greatly reduced pressures, the viscous effects of jet air flow must be taken into account. Thick boundary layers build up rapidly, tending to make the collection process less efficient than that of a comparable system with potential flow. These effects are normally associated with higher altitude ranges than will be considered here.

The type of collection surface also has a large effect on collection efficiency because of problems associated with particle bounce-off and re-entrainment. The use of an oil or grease coating tends to reduce both these effects.

Many researchers have studied the collection of aerosols by jet impactors. Davies and Alyward (3) obtained numerical solutions for impaction efficiency as a function of L/D_j and K for slit jets in potential flow. Ranz and Wong (20), using simplified potential flow equations, obtained analytical solutions for the collection efficiencies of both round and slip impactors as a function of the impaction parameter. Most experimental works on impactors have been conducted at sea level pressure, but Stern et al. (27) studied the performance of circular and rectangular jets at reduced pressures corresponding to altitudes of 10 to 25 km with 0.56 to 1.17-μ diameter polystyrene spheres. McFarland and Zeller (17) tested an experimental multiple jet impactor at pressure-equivalent altitudes of 30 to 45 km with 0.055 to 0.125-μ dye particles. The impaction characteristics for this device at jet-spacing ratio of 0.53 are presented in Figure 6.

Stratospheric impactor samplers have primarily been restricted to acquiring samples for microscopy and particle-size studies; they have therefore not found extensive use in collecting radioactive material because the sampling rates of these units have been too small to process the large volumes needed for radiochemical analyses. But at altitudes above 25 km the increased particle slip factor permits effective aerosol collection with large jet diameters (approx. 1 cm) and resulting larger flows (Fig. 7). An impactor using the multiple jet principle and designed to process volume flows comparable to those of filter units will soon be test flown. In addition, there are other advantages of impactors that should be pointed out: 1) Impactors have relatively small dimensions compared to those of equivalent volume filter systems, with particles being concentrated in a smaller area on the impactor. This makes it easier to design effective closures to protect the system from contamination, and also results in lighter, more rugged units. 2) Because particles are collected on a flat, solid surface, the system should render itself well to viability studies.

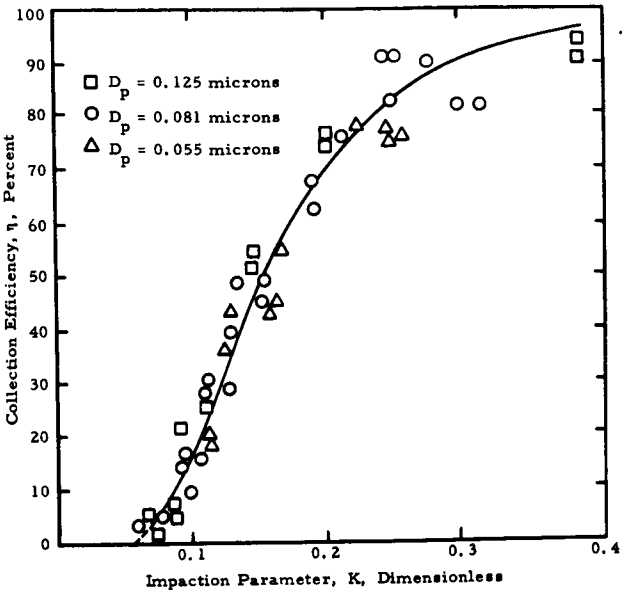

Fig. 6. Collection efficiency of a circular jet impactor at Re_j>100 and L/D_j = 0.53.

C. Other Impaction Systems

Following the nomenclature of Ranz (21), impaction systems can be categorized in two types: wall collectors and body collectors (Fig. 8). The first type is characterized by the flow of air through a system exemplified by jet impactors and tube bends. The body collector operates by air flowing around the isolated collector. (One example of this process is water droplets impacting on the body of an airplane in flight.) Conceivably either type or any

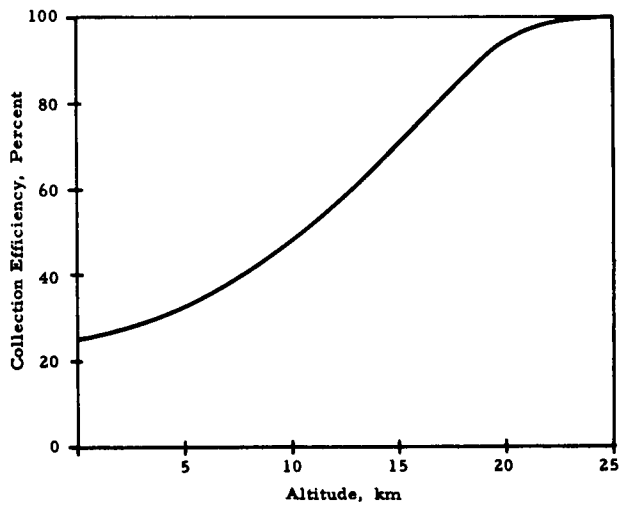

Fig. 7. Effect of altitude upon collection efficiency of a jet impactor.

variation of the two types could be used for sampling particulate matter in the upper atmosphere, but jet impactors have certain advantages over the rest: 1) As noted previously, the collected particles are concentrated in a localized area, and 2) The impaction parameter corresponding to 50% efficiency is approximately 0.1, whereas for body collectors it varies from 1 to 100.

In certain cases body collectors may be applicable. These are high-velocity sampling systems, for high altitudes at which it is much more advantageous to use a rotating body than a pump system, and close-in sampling of large particles in fresh atomic bomb debris. Most rocket-borne systems utilize the body-collecting principle. Collection efficiency characteristics of body collectors are presented in a review by Golovin and Putnam (8). At extremely high altitudes, where mean free path lengths are approximately equal to body dimensions (i.e., the collector is in free molecular flow), the collection efficiency can be determined from the work of Friedlander (6).

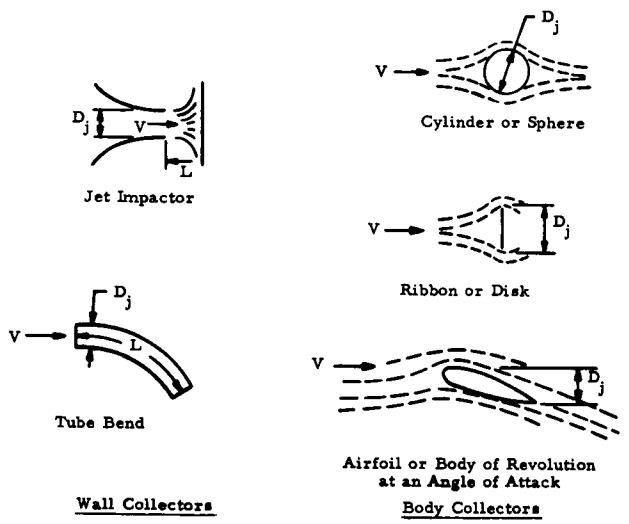

Fig. 8. Examples of impaction systems.

D. Electrostatic Precipitation

The electrostatic precipitation of particles is dependent upon two factors: the charging of particles and their motion in an electric field. A Cotrell type precipitator consists of a wire approximately 0.05 cm in diameter placed concentrically in a tube approximately 15 cm in diameter. Sufficient voltage (several thousand volts dc) is applied between the wire, and cylinder to create a corona off the wire. The escaping electrons rapidly attach themselves to gas ions in the vicinity of the wire. These in turn may attach themselves to the particles either by diffusion or by bombardment, but particles of micron and submicron size are charged primarily by the diffusion mechanism. White (33) has developed an equation for the number of charges, n, acquired by a particle as a function of time and ion concentration:

$$n = \frac{k\,T D_p}{2e^2}\,\ell n\left[1 + \frac{\pi D_p \bar{c}\, n_i\, e^2\, t}{2\,k\,T}\right] \qquad \text{XXIII}$$

where

e = electronic charge
$\bar{c}$ = root mean square ionic velocity
n_i = ion concentration.

A calculation shows that a 1.0-μ particle in an ionic atmosphere of 5×10^7 ion/cm^3 for 0.1 second will gain a charge of approximately 100 electrons.

After the particle has been charged and subjected to the electrical field between the wire and tube, it experiences a force, $\vec{F}_E$, in the direction of the field. This force is determined by

$$\vec{F}_E = q\,\vec{E} \qquad \text{XXIV}$$

where

$\vec{E}$ = electrical field vector
q = charge on particle

The force is resisted by the drag on the particles. For high-altitude sampling situations, it is expected that the particle's Reynolds number is small so that the drag force, F_D, is governed by the Stokes-Cunningham law:

$$\vec{F}_D = -\frac{3\pi\mu D_p \vec{u}}{C} \qquad \text{XXV}$$

Thus, the velocity of a particle in the electrical field is governed by

$$\vec{u} = \frac{C_q \vec{E}}{3\pi\mu D_p}. \qquad \text{XXVI}$$

For a radial field between two concentric cylinders,

$$E = \frac{\Phi}{R}\,\ln\frac{R_2}{R_1} \qquad \text{XXVII}$$

where

Φ = voltage between cylinders
R = radius from axis
R_1 = radius of inner cylinder
R_2 = radius of outer cylinder.

For this case, the radial particle velocity is governed by

$$u_r = \frac{C_q\,\Phi}{3\pi\mu D_p R}\,\ln\frac{R_2}{R_1}. \qquad \text{XXVIII}$$

The collection efficiency of an electrostatic precipitator depends upon radial velocity, which in turn depends upon charging, particle diameter, voltage, and system geometry. If other factors hold equal, it would appear from Equation XXVIII that electrostatic precipitation should be enhanced at reduced pressures. This is not necessarily the case because control of the unit becomes a problem at high altitudes where there is a difference between the corona's starting voltage, and the breakdown voltage becomes smaller with decreasing air density.

Collection of aerosols from the stratosphere by electrical precipitation has been studied by several investigators under contract from the United States Atomic Energy Commission. Liu and Whitby (16) are currently investigating the mechanisms of charging particles at reduced pressures. Langer (14) studied the development of a low-flow-rate device for the electrostatic classification of submicron aerosols. Western Precipitation (31) conducted a feasibility study in which they investigated the applicability of large-volume electrostatic precipitators for balloon-borne systems operating in the altitude range of 20 to 30 km. They concluded that such a system would have the advantages of low power requirements and light weight, but that its operation would be marginal at the highest altitudes. Del Electronics (15) has developed an experimental 4 m^3/minute collector that has been successfully tested in the stratosphere.

D. Thermal Precipitation

If an aerosol is subjected to a thermal gradient (for example, passed between a hot and cold plate), the particles move in the direction of the gradient due to the differences in the momentum transferred to the particles by "hot" and "cold" molecules. In this manner the particles are driven from the hot plate to the cold one.

Epstein (5) solved the general thermal problem for the force exerted on a particle in a thermal gradient, namely,

$$F_t = -\frac{9}{2}\pi D_p \frac{\mu^2}{\rho_a T} \frac{k_a}{2k_a + k_p} \frac{dT}{dy} \qquad \text{XXIX}$$

where

k_a = thermal conductivity of air
k_p = thermal conductivity of the particle
$\frac{dT}{dy}$ = thermal gradient.

The velocity of a particle in a thermal gradient may easily be obtained by equating the fluid drag force to the thermal force.

Using F_D given by the Stokes-Cunningham law yields

$$\vec{u} = \frac{3}{2} \frac{C\mu}{\rho_a T} \frac{k_a}{2k_a + k_p} \frac{dT}{dy}. \qquad \text{XXX}$$

Relatively high particle velocities can be obtained in a thermal precipitator. For example, 1-μ metal particles in a thermal gradient of 2,500 C/cm at 0.1 atmosphere will exhibit velocities of approximately 40 cps.

The feasibility of using thermal precipitators for high-altitude sampling systems has been investigated both theoretically and experimentally by Wilson and Orr (34). In the laboratory, they were able to attain flow rates of 0.5 m^3/minute at 0.01 atmosphere, and they propose that it should be possible to achieve sampling rates of 20 m^3/minute.

In sampling the stratosphere for biological material, thermal precipitation does not appear to offer any significant advantages over its filtration, impaction, and electrostatic precipitation counterparts at the present time. Comparatively large collection areas are required thereby giving rise to adverse signal-to-noise ratios. Also the heat radiation from the hot plate may be deleterious to viable organisms.

III. Some Current Stratospheric Sampling Apparatus

There are three means by which sampling apparatus can be carried into the stratosphere — rocket, aircraft, and balloon. Each of these three methods has in the past been used successfully for non-biological type sampling. Sampling for detection of viable microbiological particles presents additional and more severe operational criteria than have most of the past sampling requirements. Some of the equipment previously developed and now being used for sampling radioactive debris or micrometeorites would have application to certain biological sampling requirements. Several nonbiological and one biological sampler are, therefore, described in the following pages. What application, if any, this equipment would have to biological sampling depends primarily upon the concentration of biological particulates. For example, a device capable of sampling 1×10^3 m^3 of air should only be considered for use if the viable biological concentration is (or is thought to be) above this level. (Only when this need is met should other criteria even be considered.)

A. Rocket-Borne Samplers

Sampling at altitudes above 50 km is only possible through the use of rocket-borne collectors, and most of these units are still in a developmental stage. Data are included here for two units that have been used for sampling missions.

1. AIR FORCE CAMBRIDGE RESEARCH LABS VENUS FLYTRAP (23). The Venus Flytrap (Fig. 9) was developed by the Geophysics Research Directorate of Air Force Cambridge Research Laboratories for collecting micrometeorites at high altitudes. On a test flight in June 1961, the unit was carried aloft by an Aerobee 150 rocket and sampled on ascent from 88 to 168 km. To commence sampling, an electric motor moved a cylindrical shielding

section at the rear of the nose cone to the forward portion (see Fig. 9), and a second electric motor extended eight retractable leaves into the airstream.

Collection of particles on the leaves took place by the mechanism of impaction as the nose cone arose to the apogee of the flight. Shortly after the sampler started to descend, the rocket was separated from the nose cone, the leaves were retracted, and the cylindrical shielding section moved back into its closed position. At an altitude of about six kilometers, a parachute deployed.

In the flight above, four of the leaves were used for a micrometeorite penetration study, and the other four for the collection of micrometeorites for subsequent laboratory analysis. The penetration experiment consisted of exposing a 0.24 m^2 surface area of 1/4-mil Mylar film directly to the high-velocity flow field. Large micrometeorites would penetrate the film. By placing a second Mylar film a short distance behind the first, indications could be obtained of the impact energies and angles of approach.

The other part of the experiment, collection of micrometeorites, was effected by the following procedure: high-purity substrate surfaces (suitable for electron microscopy) were mounted in aluminum boxes, and two such boxes were placed on each of four leaves. The tops of the boxes were fixed to the nose-cone structure in such a way that when the leaves deployed, the box tops remained fixed to the cone, and the boxes were carried out with the leaves. After sampling, the leaves were retracted, and the boxes again sealed.

Calculations of the collection efficiency of this sampler are at best poor estimates. This is so because at the lower altitudes (88 km) the high flight velocity caused a shock wave to form at the bow of the nose cone. This complicated the flow pattern and made an accurate computation difficult. Calculations made by assuming the collector to be an isolated ribbon, however, showed that it should be highly efficient on all particles larger than 0.3 μ.

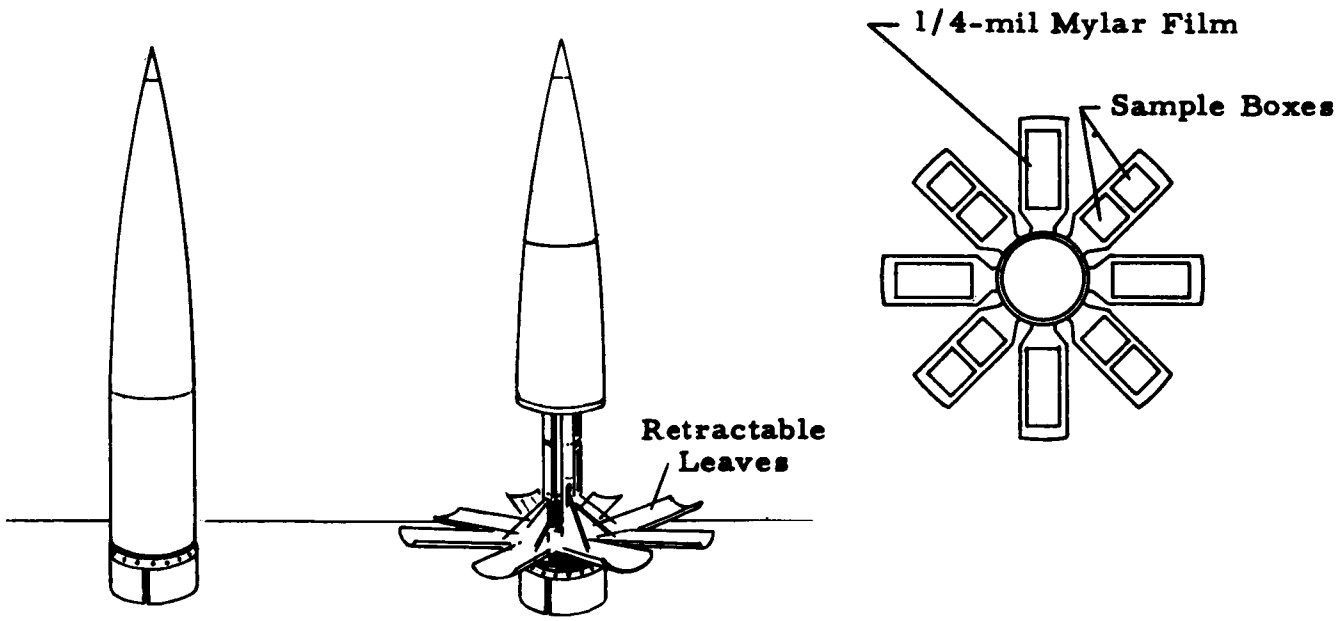

Fig. 9. Air Force Cambridge Research Laboratories Venus Flytrap. Closed position, *left*; open position, *center*; top view, *right*.

Principle of operation: Ram air operated impactor.

Carrier Vehicle: Aerobee 150 rocket.

Collection Media: 0.24 m^2 of 1/4-mil Mylar for micrometeorite penetration studies — carried on 4 leaves. 0.13 m^2 lucite plates covered with various substrates and surfaces amenable to electron microscopy studies — carried on 4 leaves in 8 sample boxes.

Operating characteristics:
1) Altitude: 88-168 km.
2) Velocity: 1.2-0.05 km/sec.
3) Volume of air swept out by projected area of collection surfaces: 2×10^4 m^3 by the 0.24 m^2 area of Mylar film. 1×10^4 m^3 by the 0.13 m^2 are of sample boxes.
4) Collection Efficiency: undetermined, but neglecting bounce off should be highly efficient on all particles > 0.3 μ.

The problem associated with a particle's temperature rise (Fig. 1) during collection must be solved before a device of this type could be used for biological sampling.

2. AIR FORCE CAMBRIDGE RESEARCH LABORATORIES NOCTILUCENT CLOUD PARTICLE SAMPLER (24). Particle sampling experiments utilizing Nike-Cajun sounding rockets were conducted in northern Sweden during the summer of 1962. These tests, conducted jointly by scientists of the United States and the University of Stockholm, Sweden, utilized a sampler having the external configuration shown in Figure 10. Two successful flights were made, one in the presence of noctilucent clouds and one when no such clouds were observed. The collection surfaces were exposed between altitudes of approximately 75 and 95 km during ascent only.

To begin collection at sampling altitude, specially prepared collection surfaces were exposed by spring ejection of an outer rocket nose tip. These sampling surfaces were mounted in cylindrical containers.

On completion of the sampling period (while the rocket was still ascending), a second spring mechanism closed the sampling ports and at the same time covered and sealed the sample containers. Since orientation of the sampler in the direction of flight is important, samples were not taken during descent.

On the two successful flights over Sweden, the rocket aimed at visible noctilucent clouds collected some 2 million particles/cm^2 of collecting surface. This was between 100 and 1,000 times more than the number of particles collected by an identical probe which traversed the same path on a cloudless night. The size of solid particles collected ranged from 0.05 to 0.5 μ in diameter.

At sampling altitude, the collector and particles were in a region of free molecular flow and it may be assumed (neglecting the possibility of particle bounce-off) that the collecting surface swept these particles from a projected area volume with 100% efficiency. With respect to the tests reported, particles were swept from a volume of approximately 30 m^3. Work is continuing and plans include collector modifications that will eventually permit measurement of particle size and concentration as a function of altitude.

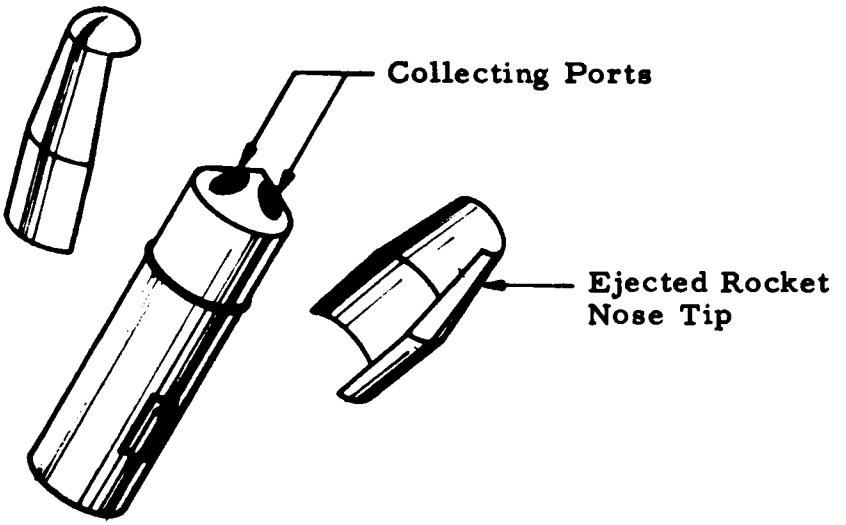

Fig. 10. Air Force Cambridge Research Laboratories noctilucent cloud particle sampler.

Principle of operation: Ram-air operated impactor.

Carrier vehicle: Nike-Cajun rocket.

Collection media: 1) Nitrocellulose film with evap. alum. coating. 2) Nitrocellulose film coated with fuschine dye. 3) Indium film. 4) Calcium film with coatings of aluminum, paraffin and silicone oil.

Operating characteristics:
1) Altitude interval: 75 to 95 km.
2) Velocity: 0.9-0.6 km/sec.
3) Volume of air swept out by projected area of total collection surface: approximate 30 m^3.
4) Collection efficiency: neglecting bounce off, should be virtually 100% for particles 0.05-0.5 μ diameter.

Although the Air Force Cambridge Research Laboratories' Nike-Cajun sampler collects particles from a rather small volume, it does represent a well-considered effort in which the designers have accepted a few necessary compromises in order to obtain a reliable, lightweight research tool. For biological sampling, the sampled volume is much too small and the problem of heating is ever present.

B. Aircraft-Borne Samplers

1. U-2 HATCH SAMPLER (7, 25). The U-2 hatch sampler is a ram-air-operated sequential filter collector. The sampling package (Fig. 11) is a removable foil fitted to the underside of the aircraft just behind the pilot compartment. The sampling duct and filter being exposed are located outside the aircraft body, whereas the filter changer and the other associated apparatus are inside. Entrance to the sampling duct is a 12 cm x 21 cm elliptical opening that is fitted with an internal butterfly-type closure door. The duct expands and changes from the elliptical opening to a 40-cm diameter circular cross-section at the filter location. Six IPC 1478 filters can be carried aloft during each flight. They are stacked in the closed filter-disk housing and are inserted into the sampling duct by the changing mechanism upon command from the pilot. Each 40-cm diameter filter is mounted between two screens and supported in the back by a cast metal stiffener.

Sampling rate for the hatch sampler is about 100 m^3/minute. Collection efficiency at 20 km altitude and 215 m/second (415 knots) flight velocity would be > 90% for all particles over 0.1-μ diameter.

The U-2 sampler in principle could be used to advantage for biological sampling; several modifications would be necessary to protect the biological integrity of this collected sample and to prevent accidental contamination.

2. ISOKINETIC CASCADE IMPACTOR PROBE (29). The isokinetic cascade impactor probe was developed for Air Weather Service WB-50 aircraft flying routine missions at 500 mb pressure altitude and 120 m/second (235 knots) air speed. Two of these units have been in service for over a year and have been used for sampling aerosols when the aircraft are not flying in clouds.

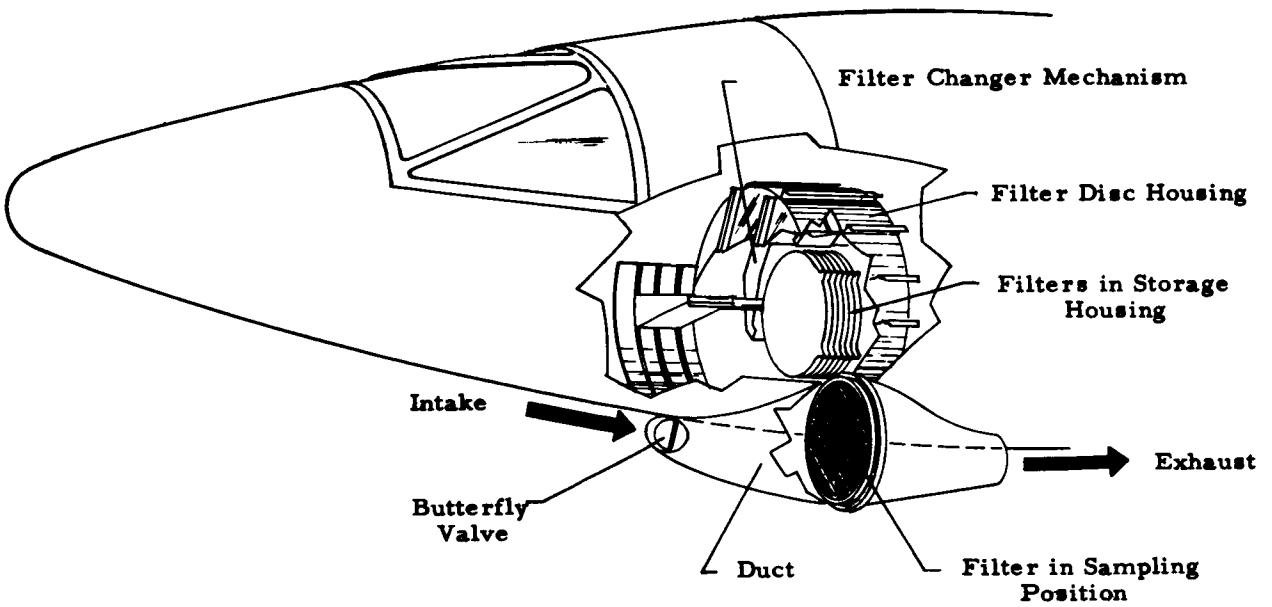

Fig. 11. U-2 Hatch Sampler.

Principle of operation: Ram air operated filter collector — as many as 6 filters may be exposed sequentially during a single flight.

Sampling media: IPC 1478 filter paper.

Filter area: 0.13 m^2.

Sampling rate: 10 m^3/min at a flight velocity of 225 m/sec and altitude of 20 km.

Allowable sampling time: up to 6 hr.

Altitude range: < 20 km.

Collection efficiency: >90% for D_p >0.1μ.

This unit has two impaction collection stages followed by a filtration stage (Fig. 12) that utilizes IPC 1478 filter paper. Particle size cutpoints (particle size at which collection efficiency is 50%) of the impactor stages are 2 and 0.3 μ, based on a particle density of 2.0 g/cm^3. Design sampling rate of this device is about 0.4 m^3/minute of ambient air. It is capable of operating on extended flights of up to 10 hours.

Collection surfaces for the impaction stages consist of grease-coated glass slides, which are mounted in removable slide holders designed to give accurate alignment of the slides with respect to the impactor nozzles. Because the second-stage nozzle is quite small, a rotary slide is used to present a greater area for collection. This is actuated by a ratchet that rotates the slide at the rate of 1/8 rpm.

The sampling probe is mounted on the nose section of the WB-50 fuselage. At this location, the local air velocity and direction are slightly different from that of the free stream; the local velocity is about seven percent higher and the direction is essentially parallel to the aircraft surface. These conditions dictate both the sampling velocity and the probe alignment if the principles of isokinetic sampling are followed. For this sampler, however, isokinetic conditions were attained by a cowling at the probe's inlet. This has two advantages: 1) the inlet airstream can be decelerated to the velocity required in the first-stage impactor nozzle, thereby eliminating the necessity for an inlet diffuser that could impair the impactor's collection characteristics, and 2) a uniform-flow field is created within the shroud that keeps the curvature of the inlet flow lines small, preventing particle discrimination. Although the velocity within the cowling is less than the free stream velocity (50 m/sec compared with 120 m/sec) the streamlines in the center of the cowling are straight, and it is these that lead to the inlet of the sampler.

Because the WB-50 aircraft does not generate sufficient ram pressure to permit isokinetic sampling, an inboard pump must be used. Flow into the pump is adjusted by a throttling valve to match the inlet velocity in the first-stage nozzle with the air velocity in the shroud. The flow rate through the system, obtained from measurements of the pump's inlet pressure and temperature, is accurate to within ±5%.

An external closure door is used to prevent contamination during non-sampling periods (which includes the time the plane is flying through clouds). During sampling, the door is drawn up and recessed in the cowling. The door recess and the outside face of the closure door are electrically heated to prevent icing. In addition, the first-stage nozzle is heated to lower the degree of saturation of the sample air, thereby preventing condensation from occurring within the sampler's body when the ambient air is very humid, but has not reached the stage of cloud formation or precipitation.

The unit is controlled by the weather observer, but data such as elapsed time, pump inlet pressure, and temperature are recorded photographically from an instrument panel.

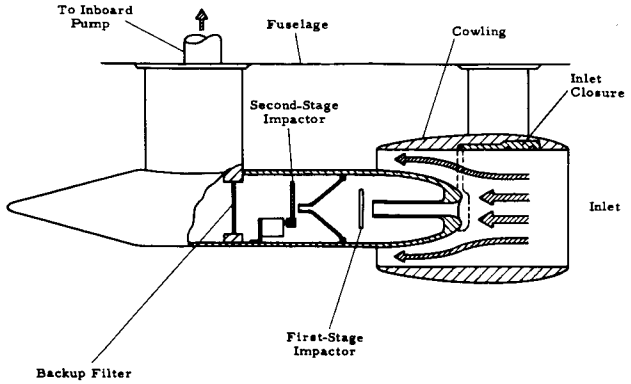

Fig. 12. Isokinetic cascade impactor probe (WB-50 aircraft particle sampler).

Operating conditions
Altitude: 5.4 km (500 mb)
Air speed: 120 m/sec
Flow rate: 0.4 m^3/min ambient air

Design characteristics	1st Stage	2nd Stage
Particle size cutoffs, μ	2.0	0.3
Nozzle velocity, cm/sec	4,900	18,700
Nozzle diameter, cm	1,365	0.778
Filter	IPC 1478	
Filter diameter, cm	4.5	
Filter face velocity, cm/sec	600	

The principal disadvantage of the impactor probe for biological sampling is low flow rate.

C. Balloon-Borne Samplers

As sounding vehicles for stratospheric sampling systems, balloons are the "old stand-bys" that now provide a capability for lifting relatively large payloads to altitudes as high as 45 km and can keep them there for hours or days with the expenditure of little energy. Balloons are being used in continuing programs by the Atomic Energy Commission, the Air Force, and other agencies in connection with monitoring the world-wide inventory of residual bomb debris in the stratosphere.

1. NATIONAL AERONAUTICS AND SPACE ADMINISTRATION BIOLOGICAL SAMPLER (9, 10, 35). This balloon-borne unit (Fig. 13) was developed to collect microorganisms from the stratosphere. In physical size and construction the unit is similar to the Atomic Energy Commission direct-flow samplers: it is about 0.7 m in diameter x 1.3 m long, and is fabricated entirely from aluminum. Sampler skin and inlet cone are aluminum spinnings and the frame is aluminum tube. Each unit weighs approximately 25 kg (without associated apparatus). A blower is used to pull air through a filter, and the flow volume of sampled air is monitored by an anemometer-type flowmeter located at the discharge of the blower.

Four samplers, mounted at the corners of the gondola, are carried aloft during each flight. Total weight of the package is about 300 kg. Normally one sampler is used for control purposes and three are used for actual sampling Prior to flight the four assembled samplers are autoclaved at 125 C for 1 hour to kill off background contamination. The inlets and outlets are covered with jettisonable aluminum dust covers that protect the unit when it is removed from the autoclave. In addition, jettisonable nylon shrouds are used on the inlets. The spring-loaded valve closure is in the open position and remains so until sampling is completed. After autoclaving, the samplers are mounted on the gondola and the complete sampling package is enclosed in a plastic shroud. Ethylene oxide is pumped into the shroud to kill off surface contamination on the gondola and samplers. Just before take-off, the shroud is removed and spring-loaded closure door of one of the samplers is released. It slams shut and locks in the closed position. This sampler provides a ground control. The system is designed to sample during the descending portion of the flight, so the balloon carries the package to maximum altitude, at which point the jettisonable dust covers and nylon shrouds are released and parachuted to earth. Simultaneously, sufficient helium is vented from the balloon to permit a controlled descent rate of about 150 m/minute. Shortly after descent starts, two samplers are energized. The first one samples for a short period (about 3 min), is shut off, and its spring-loaded closure door is released. The sample collected by this unit serves as an in-flight control. The other unit samples through a preprogrammed altitude range (for example, from 27 — 18 km); then it too is shut off and sealed. The remaining unit samples over a second altitude increment (for example, from 18 — 12 km). The balloon continues on its descent until the sampler impacts, whereupon it is cut loose to avoid dragging the payload. Each sampler body has a small pressure-equilibrium port fitted with an absolute filter so that during ascent and descent only sterile air leaks into the unit.

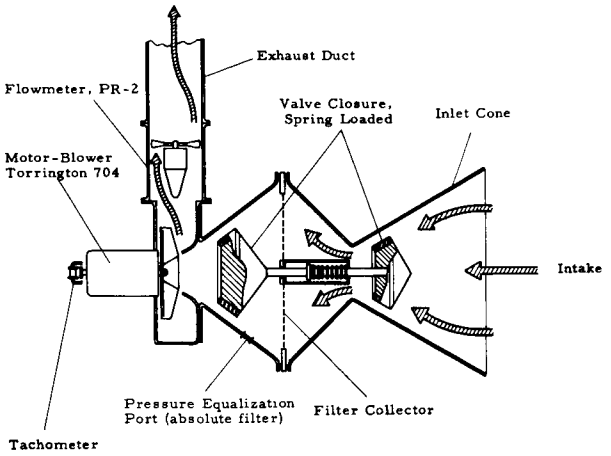

Fig. 13. National Aeronautics and Space Administration biological sampler (balloon-borne).

Weight:
Sampler, 25 kg.
Batteries & aux. equip., 50 kg.

Blower: Torrington 704, 0.52 hp.

Filter:
(biological sampling), Polyurethane foam, 80-pore, 0.09 m^2.
(radioactive particulates), IPC 1478 paper, 0.09 m^2.

Sampling rate:	Poly filter	IPC Paper
18 km	30 m^3/min	20 m^3/min
25 km	30 m^3/min	15 m^3/min
30 km	...	10 m^3/min
35 km	...	3 m^3/min

Volume determination: from PR-2 flowmeter and/or blower rpm.

About 0.09 m^2 of 1.3 cm thick 80-pore polyurethane foam (Scott) is used as a filter medium for the biological sampling missions. Other media (such as IPC 1478) would also be compatible with the mechanical system.

For sampling below 30 km altitude with a balloon-borne sampler, the National Aeronautics and Space Administration unit concept is good. Several modifications to the existing system could, however, be made to decrease the contamination possibility both before and after sampling.

2. ATOMIC ENERGY COMMISSION EJECTOR POWERED IMPACTOR SAMPLER (17). This unit represents the first attempt to sample large volumes of stratospheric air by a jet impactor. In the past, when sampling systems were limited to altitudes of less than 30 km, the flow rates obtainable with submicron impactor collections were small. Heavy pumping systems were required to provide the large pressure drop needed for accelerating the air at the impactor nozzles to high velocities. As altitude capabilities were extended above 30 km, it became apparent that impactors could be used to advantage. Significantly, at these altitudes impactors do not suffer from their usual deficiencies at lower heights. The particle slip factor becomes extremely large, thereby permitting the effective collection of submicron particles with large (centimeter size) low-velocity jet systems. In addition, flow rate can be increased through the use of multiple jets to compare with that of contemporary filter units, and the pressure-drop characteristics of the sampler are compatible with the lightweight air-ejector pumping system.

The sampler has two impactor stages followed by a filter stage (Fig. 14). The first stage is designed to have 19 nozzles 1.8 cm in diameter and it will be 50% efficient at 0.3 μ. The second stage has 49 nozzles 0.7 cm in diameter and will be 50% efficient for 0.03-μ diameter particles. The sampling rate is 15 m^3/minute for 100 minutes. The filter paper presently being used is IPC 1478.

The samples are protected on both the upstream and downstream sides by closure doors. With the exception of the sampling period, these doors are sealed from the time the sampler is assembled in the laboratory until it is returned for disassembly after the mission has been completed.

The air ejector-impactor could be used for biological sampling; however, the second impactor and back-up filter would serve no useful purpose in collecting micron-sized particles. A variation of this same unit has been flown for the Atomic Energy Commission with only a filter (no impactor stages) and this form should be well suited for biological sampling. Either a balloon-borne air ejector filter or air ejector single-stage, multiple-jet impactor would provide an extremely attractive sampling package for biological sampling in the 25 to 45 km altitude range. Sampled volumes of 10^4 m^3 should be possible with this type of sampling system.

Acknowledgment

This work was conducted under the auspices of the U. S. Air Force, U. S. Atomic Energy Commission, and National Aeronautics and Space Administration.

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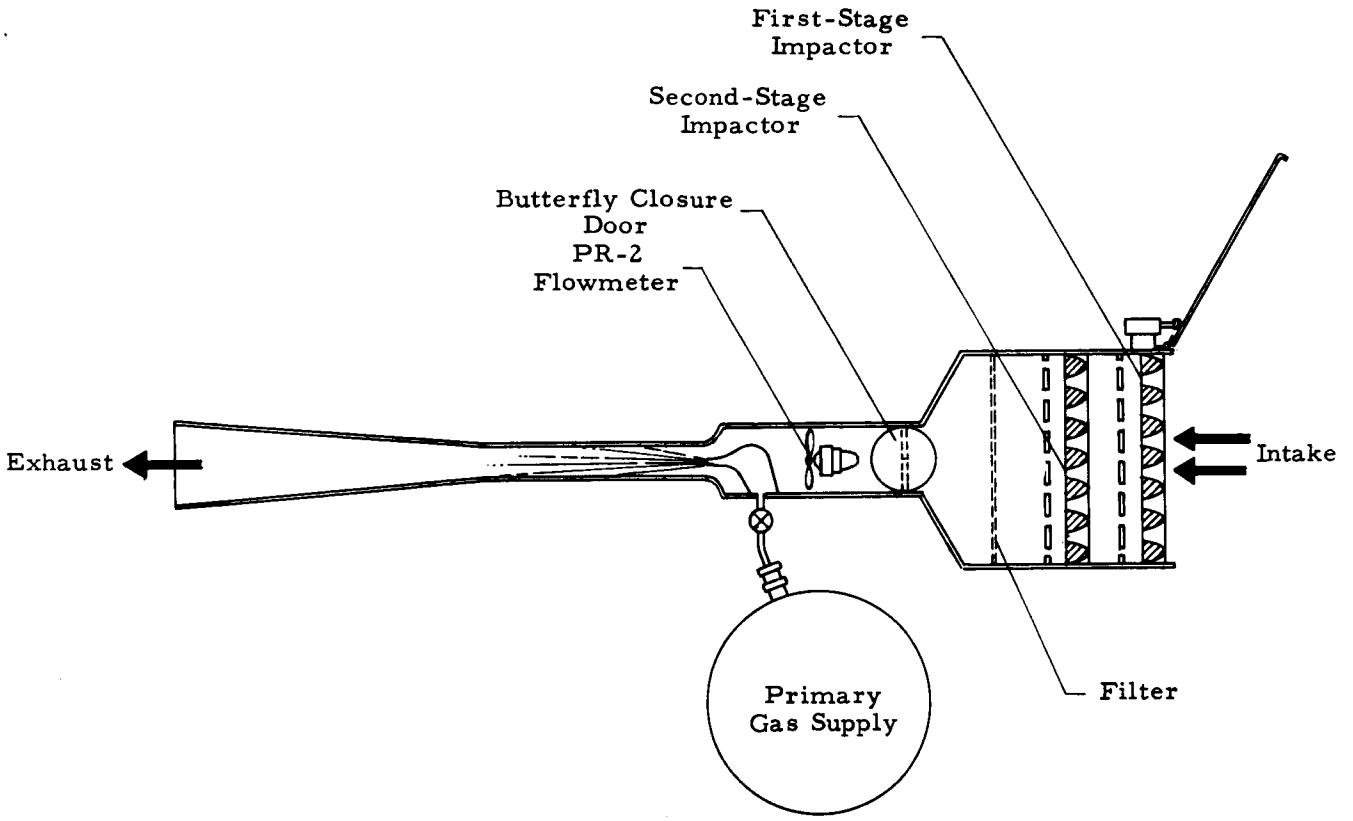

Fig. 14. Atomic Energy Commission air ejector powered impactor sampler (balloon-borne).

Weight: (sampler & aux. eqpt.) 100 kg.

Operating conditions
Altitude: 25-45 km
Sampling rate: 15 m^3/min ambient air
Allowable sampling time: 50-200 min

Design characteristics	1st Stage	2nd Stage
Cutpoint	0.3 μ	0.03 μ
Number of jets	19	49
Jet diameter	1.8 cm	0.7 cm
Jet velocity	5000 cm/sec	16,200 cm/sec

Volume determination: PR-2 flowmeter

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Discussion

Phillips — I got lost in your surface heat figures for rockets, high-performance crafts, or something like that. Are you talking about the temperature of a compressed air film?

Lundgren — Just from thermodynamic considerations, if you consider the temperature rise resulting from accelerating a particle from zero velocity to the flight velocity of the vehicle, isentropically, the plot would give this temperature rise as a function of the flight velocity.

Phillips — This would be a temperature to which the microorganism would be exposed for a fraction of a second?

Lundgren — This would depend upon the collection system. If the microorganisms were impacted out on the frontal surface of a collector, and this collector were continually exposed to the air stream, then the entire frontal surface would approach this temperature; the organism would then be exposed to this temperature for a long period of time.

Phillips — Yes, but usually one is bringing things back inside some kind of a collecting device.

Lundgren — Right. There is a heat transfer problem involved here. The particle may not attain this temperature. It would take time to do so. A gas molecule would attain this temperature, but how much of this heat would be transferred to the internal part of the organism?

Phillips — I really only wanted to make the comment that the situation might not be quite as pessimistic as you think. The lethal effect of heat has not only a temperature, but a time factor. It's not only the temperature one attains, but the time of exposure and the resistance of microorganisms to extremely high temperatures. One can mix organisms with nitrocellulose, explode the nitrocellulose, and obtain sizeable numbers of viable microorganisms in aerosol form. The explosion temperature is high, but the time is correspondingly short, and all cells are not killed.

Lundgren — Dr. Greene has some data on the effect of time, temperature, and viability of lyophilized *Serratia marcescens* and *Bacillus globigii*. In his experiments the bacteria were exposed to temperatures of 70 to 200 C for 0.5 to 1.68 seconds. *S. marcescens* showed a log decrease when subjected to 125 C for 1.68 seconds where the hardier *B. globigii* withstood 175 C for the same time period before showing a tenfold viability reduction.

Phillips — These times are still long when compared to times of passage through the high-temperature gas phase in an explosion.

Lundgren — The problem is preventing the organisms from being collected in a position or by a device that would subject the organism to this temperature for a long period of time. With reasonable time periods this temperature would have serious effects, but if there were some method by which the organism could be moved rapidly through this high temperature area it would be a different problem and one which probably could only be answered by experimental work.

Gregory — I have two questions. I am interested in your remarks on interception. Does this have any practical significance? It looks like a small effect. The particle would be in a position to receive maximum blast from blow off on the edge of the fiber? Question two is, have you any data on the retention of viability by organisms exposed to a fast-moving air stream on the surface of the filter paper or mass of fibers? There they are not quite in the same position, not quite in the same environment, as they are when in suspension in air.

Lundgren — We have some results from the decrease in viability of lyophilized organisms collected on polyurethane foam. As I recall, there was no significant decrease in viability when sampling from 10 to 1,000 minutes with sampling velocities on the order of 1,000 ft/minute. There is a velocity effect, but not at the conditions obtained with the balloon-borne samplers or at the velocities encountered.

You mentioned blow off. When you consider the forces holding a micron-size particle to a surface, you'll see that the blow off is extremely low because the adhesive forces are quite high. The force one can get on a micron-size particle with anything much less than sonic velocities would not tend to blow it off, whereas with a particle of 10 to 100 μ diameter, the case is quite different. Then the particle can be blown off or be re-entrained in the air stream. Another factor is bounce off. Even a millimicron-size particle hitting a surface at high velocity may bounce off. Bounce off can be defined differently from re-entrainment by a time factor. We have conducted some low pressure bounce-off studies using impactors. An adhesive is needed when the jet Reynolds number in the impactor is greater than a thousand or so. (For Reynolds numbers less than a hundred an adhesive is not required.) The required adhesive thickness is a function of the particle size. An adhesive that is several times thicker than the diameter of the particle is sufficient to prevent bounce off.

Ranz — I would like to ask the speaker if he can give us some information about how fast the particles hit. Perhaps some of our friends in the audience could tell us about how fast the particles can hit and still live.

Lundgren — That's a good question, but I have no data to present. This, again, would be a limitation to high-speed sampling. I can imagine a biological particle being shocked in some way by a rapid deceleration from Mach 2 down to zero. What effect this has I don't know, but it sounds like it should have a rather drastic effect.

Phillips — Some experimental data touch on this. Ordinary impactor samplers operating at room temperatures, for example, cause air to be drawn through at sonic velocity, thus impacting particles on hard surfaces at somewhat slightly less than sonic velocity. Thus at a hard impaction just under Mach 1, there's almost 100% survival of bacterial spores. With vegetative microorganisms the percentage of survival will decrease according to the fragility of the organism. There's seldom 100% survival; with some organisms there will be almost zero survival. With more resistant organisms like staph, there could be as much as 50% survival.

Lundgren — I assume the organisms in the stratosphere tend to be of the more hardy types you mentioned rather than of the less hardy types.

Phillips — Well, there's a fantastic range in hardiness among vegetative bacteria. You can't give one figure just for vegetative, and another for spores as to what happens upon impaction. However, almost all of the bacterial spores and many of the fungal spores can survive.

Detection and Study of Microbial Population in Upper Atmosphere

Simulated High Altitude Microbial Sampling With an Electrostatic Precipitator

David Nicholson, William J. Oswald, and Robert C. Cooper [1]
University of California
Berkeley

Abstract

In our study of microorganisms that might exist in the upper atmosphere, a method of capturing such microorganisms in viable form is required. Theoretical studies indicated that a high collection efficiency could be attained with an electrostatic precipitator under near vacuum conditions.

Experimental verification of the theoretically high efficiency of the precipitator and of the viability of organisms captured was sought through use of a laboratory aerosol chamber. In the chamber, suspensions of *Serratia marcescens* were established with atomizing equipment. An air stream drawn from the chamber was passed through the precipitator and through an Andersen sampler in parallel. The collected material was regrown and enumerated to evaluate the collector efficiency as compared with the Andersen. Many more viable organisms were collected in the Andersen than in the precipitator. With a second Andersen in series following the precipitator in the air stream, it appeared that the precipitator removed many more bacteria than could be enumerated as viable. On the basis of the limited evidence presented, it is tentatively concluded that *S. marcescens* was to a large extent killed during collection in the electrostatic precipitator. The killing mechanism (perhaps ozone production in the corona discharge of the precipitator) is under study. Studies are also in progress to modify the precipitator to increase its efficiency.

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With development of the science of microbiology and of methods for detecting microbial cells, the ubiquity of microbial life is well established. Microbes abound in the soil and water of the earth and pervade its atmosphere. In the air near the earth's surface there are usually more than a million microbial cells per cubic meter and during disturbances such as dust storms this number may increase by several orders of magnitude. To what elevation above the earth can microbes be carried? Does microbial life exist at 50, 100, or 200,000 ft or beyond? If such life could be found in the upper atmosphere, could it originate from the surface of the earth, borne upward by violent winds? If so, then is this life carried by winds only to the limit of the dust sphere below 37,000 ft? Or is microbial life carried upward by volcanic eruptions or atomic bomb explosions?

Could microbial life exist in the upper atmosphere because it pervades the universe?

If windborne, one might expect to find a normal lapse rate for microbial life similar to the normal lapse rate for pressure with elevation. On the other hand, if microbial life continuously enjoins the atmosphere from outer space, one could perhaps expect increased numbers of microbes in the upper atmosphere during meteor showers or during interception of one of the many comets by the earth's orbit. If one were to find that there is no true ceiling for microbial life in the atmosphere, it should follow that microbial spores could occasionally attain escape velocities, thus entering a pan spermatic population.

Since the studies of Walker (8) and of Rogers and Meier (7), it has been the objective of many investigators to chart the profile of microbial occurrence in the upper atmosphere. In spite of these many efforts, however, the microbial profile remains undefined.

One explanation of our inability to define a profile is that none exists. The work of Greene (2) indicates, however, that microbes occur up to 90,000 ft.

Another explanation of our inability to define a profile is the possibility that microbial forms are not detected because they are killed by the rigors of the upper atmosphere. The fact that microbial forms are capable of withstanding the conditions in the upper atmosphere is indicated by the work of Greene, and by that of Morelli (5), who has demonstrated that *Bacillus subtilis* can withstand a vacuum of 10^{-8} mm Hg for 35 days. This pressure corresponds to that in the atmosphere well above 100,000 ft. High temperatures that exist in the upper atmosphere may be lethal to microbes; the effects of high temperatures at the low pressures that are present in the upper atmosphere are unknown. Reported concentrations of ozone in the upper atmosphere approach the 0.2 ppm which was indicated by Elford (1), to be lethal to moist bacteria deposited on surfaces, but little is known of the permeability of ozone into spores at reduced pressures. Hence, we conclude that no ceiling is clearly imposed by environmental conditions in the upper atmosphere.

The major difficulty in establishing a microbial profile in the atmosphere is the simple lack of a sufficient number of reliable samples. Our program at the University of California has therefore been oriented toward the development of versatile, inexpensive, and reliable microbial sampling systems. Our efforts to date have been centered upon developing these systems in the laboratory prior to undertaking a high altitude sampling program. Our objective is to develop a sampling system versatile enough to be used with any type of conveyance, be it balloon or artillery shell. We hope to develop a system so inexpensive that a large number of samples can be taken at relatively low cost. We hope to develop a sampler in which asepsis is easily maintained and one that will trap a sufficient number of microbes for dependable results. We have not put our entire efforts into any single system.

Our selection of a preliminary system was based upon several criteria. In the first place, a device was sought which will efficiently collect particles about one micron in diameter, the assumed most probable diameter of high altitude microbes. In the one-micron range of particle size, according to McCabe (4), any collection mechanism is theoretically in the transition zone between utilizing the effects of inertia and those of diffusion.

Deflection, impaction, and filtration devices did not appear ideal for a number of reasons. For example, impactors could conceivably kill microbes by conversion on impact of kinetic energy to heat energy. The same problem could arise in the use of deflectors. Filtration, on the other hand, poses severe limits on the volume of atmosphere that can be sampled. The device which apparently overcomes most of these objections and meets nearly all of our specified design criteria is the electrostatic precipitator. Electrostatic precipitators have been used by various people for air sampling as well as for commercial and domestic air cleaning. Various modifications of the basic concept exist; for example, a concentric-cylinder version was described by Houwink in 1959 (3). It was decided therefore to investigate the electrostatic precipitator as a potential device for sampling bacteria in the upper atmosphere. The special advantages of an electrostatic precipitator are 1) theoretically high efficiency for collecting particles at high flow rates and 2) low resistance to high volumes of air flow.

Methods

The theoretical equations of White (9) (Fig. 1) were used as a basis for design of the precipitator system to be evaluated. In its present form the electrostatic precipitator tubes have an inside diameter of 2 inches, a wall thickness of 1/8 inch, and a length of 13-5/8 inches. Two materials have been tested for the precipitator tube. Aluminum is used when the recovery technique does not require culturing the organisms inside the tubes. Pyrex glass coated with stannic oxide for electrical conductivity is used when the technique requires culturing directly in the tube. The aluminum inner electrode is 3/4 inch in diameter with a hemispheric tip from which extends a tungsten discharge wire 0.016 inch in diameter and 1/2 inch long (see also Fig. 2). From White's equation and the precipitator dimensions, theoretical variation in efficiency of the precipitator with particle size was computed. The precipitator theoretically attains an efficiency of 98%

$$\eta = 1 - e^{-\frac{A}{V}W}$$

where η = efficiency of electrostatic precipitator
A = collecting area, ft^2
V = volume flowrate, ft^3/sec
W = precipitation drift velocity, ft./sec

$$\frac{E_o E_p a \gamma c}{2\pi\Theta(30.4)}$$

E_o = average charging field, statvolts/cm
E_p = average collecting field, statvolts/cm
a = particle radius, cm
γ = fractional particle saturation charge
Θ = air viscosity in poises
$c = 1 + \frac{.86 \times 10^{-5}}{a}$

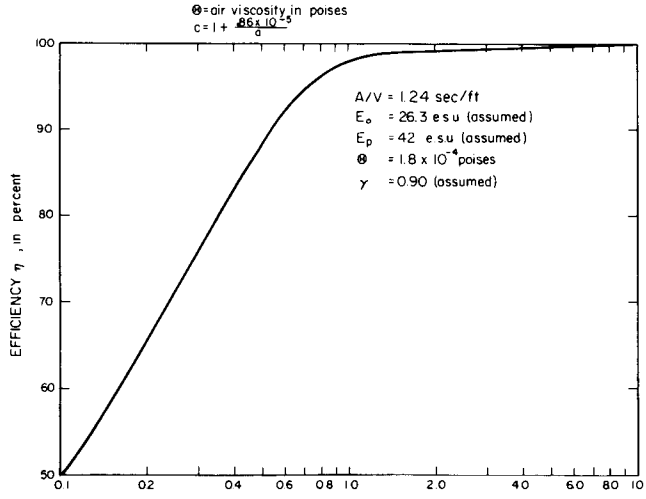


FIG. 1. THEORETICAL EFFICIENCY EQUATION FOR ELECTROSTATIC PRECIPITATOR (according to White, 1960)

FIG. 2. EFFICIENCY VERSUS PARTICLE SIZE, BASED ON $\eta = 1 - e^{-\frac{A}{V}W}$ WITH $\frac{A}{V}$ CONSTANT

for particles of 1 μ in diameter when conditions are as stated (Fig. 2).

The precipitator, ready for an experimental run, is shown in Figure 3, mounted in a special test holder. The glass outer cylinder is shown. During collection of samples, discharge of ions from the thin electrode tip ionizes such air molecules as may be present. The charged air molecules then intercept particles which enter the field. As a collectable particle enters the field it becomes charged and passes to the outer electrode. Following a run the collection tube may have viable microbes on its surface.

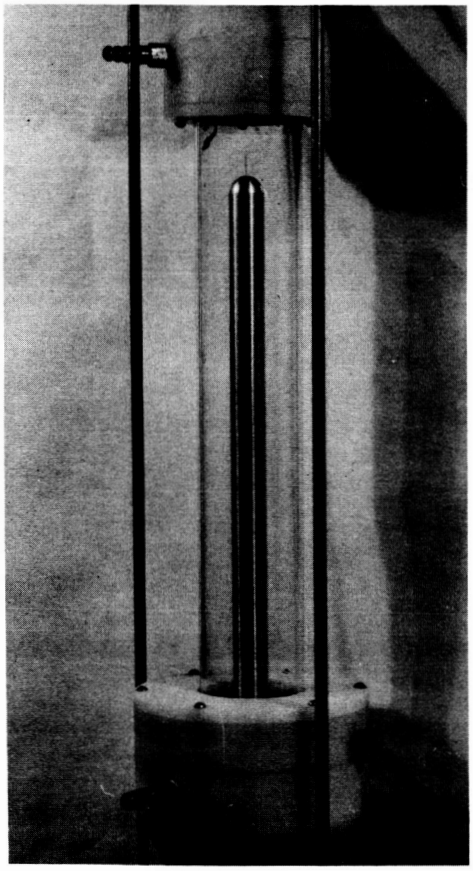


Fig. 3. Electrostatic precipitator in test position. Glass barrel is normally replaced with aluminum outer electrode.

To experimentally calibrate the electrical properties of the precipitator under atmospheric conditions (and eventually under low pressure conditions to simulate the upper atmosphere) a special test system was devised (see block diagram, Fig. 4). In performing the calibrations the electrostatic precipitator (cf. Fig. 3) with a 1-inch discharge wire was adjusted to the desired internal pressure and flow rate. Variable high voltage was then

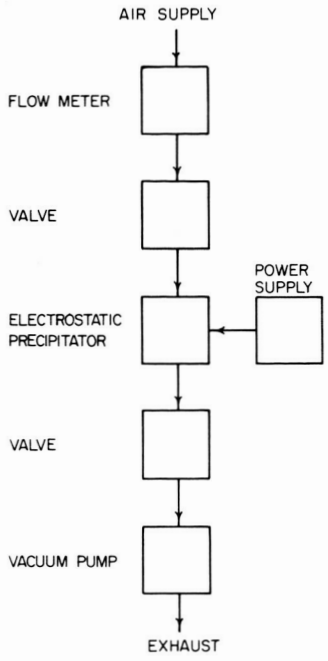


FIG. 4. BLOCK DIAGRAM OF TEST FOR ELECTRICAL CHARACTERISTICS OF THE ELECTROSTATIC PRECIPITATOR AT LOW PRESSURE

applied throughout the applicable range of pressures and flow rates and recorded giving both data on calibration tests for voltage and current (Figs. 5-8, incl.).

In Figure 5 the relationship is shown between current and voltage in the precipitator with an airflow of 1 scfm at atmospheric pressure for both positive and negative corona discharge. The current in the precipitator (Fig. 5) increased rapidly with increased voltage between 2 and 10 kv for negative corona and between 4 and 11 kv for positive corona.

In Figure 6 are shown the results of tests carried out to calibrate the voltage versus pressure relationships for the precipitator. At low pressures (below 1 inch of Hg abs.) little voltage was required to maintain a current of 150 μ amps when air flow was 0.39 scfm.

In Figure 7, a calibration is presented of current versus pressure for a constant air flow of 1.3 scfm and 5.0 kv. Current decreased with increasing pressure.

In Figure 8 results are presented on calibration of current versus air flow for constant air pressure and precipitation voltage. Current was unaffected by air flow.

Having calibrated the electrical properties of the precipitator we next undertook a series of simplified tests to evaluate the effectiveness of the

electrostatic precipitator in collecting a known microbial aerosol at atmospheric pressures (see block diagram, Fig. 9). An aerosol chamber was used as a reservoir for microbial cells (see foreground of Fig. 10; for identification of other portions, see Fig. 9). An Andersen sampler (Andersen Sampler and Consulting Services, Provo, Utah) used in series with the electrostatic precipitator is not shown in Figure 10.

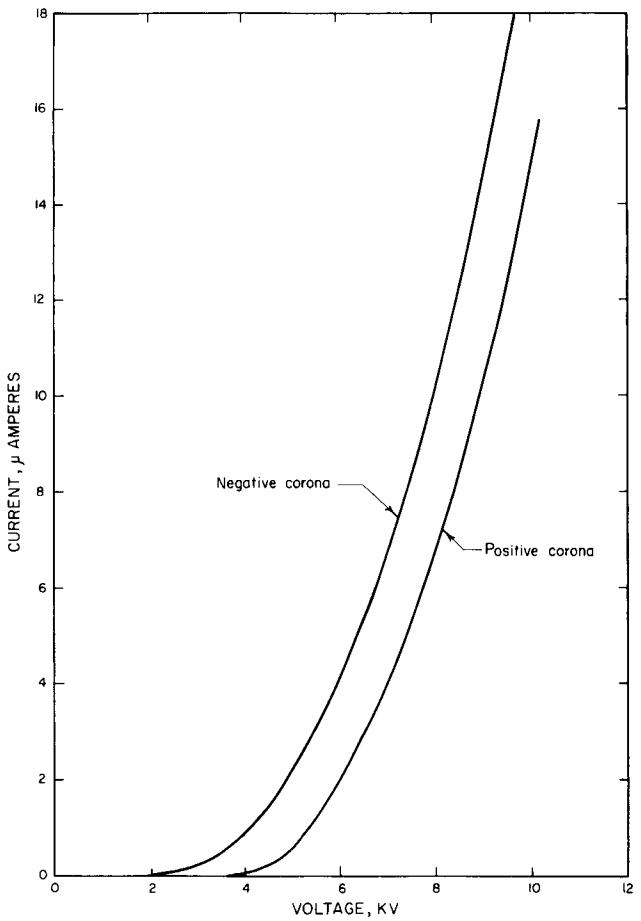


FIG. 5. CURRENT-VOLTAGE RELATIONSHIPS FOR POSITIVE AND NEGATIVE CORONA AT ATMOSPHERIC PRESSURES WITH 1 SCFM FLOW RATE

The experiments delineated (see diagram, Fig. 9) were carried out primarily to compare the experimental efficiency of the precipitator with that of an Andersen sampler in parallel. A second Andersen sampler was used following the precipitator to determine the number of organisms escaping the precipitator and caught by the Andersen.

During a typical evaluative test a wet inoculant of *Serratia marcescens* was introduced into the aerosol chamber utilizing a vaponefrin nebulizer as an aerosol generator. *S. marcescens* is typified by a

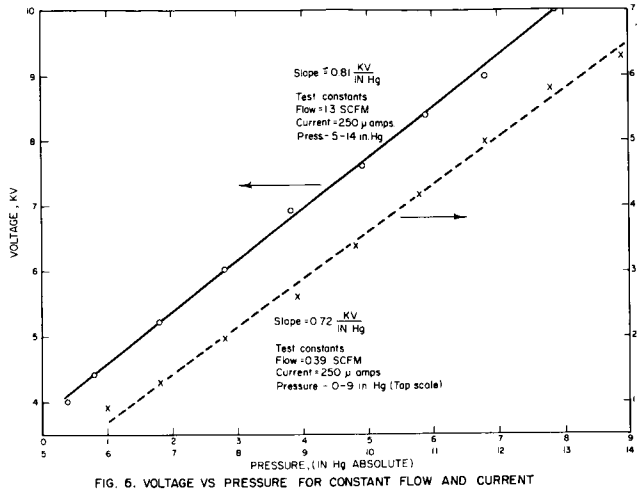
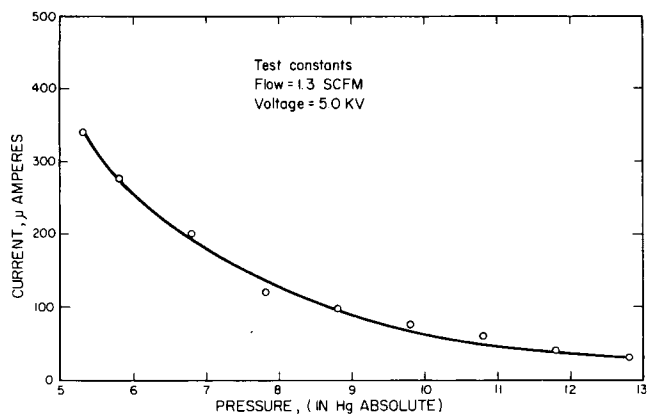


FIG. 6. VOLTAGE VS PRESSURE FOR CONSTANT FLOW AND CURRENT

characteristic red color when cultured on certain nutrients, making it easily identifiable in spite of the occurrence of contaminating microorganisms. The chamber was pressurized to 5 psig to create a positive pressure differential which would then provide adequate flow through the precipitator.



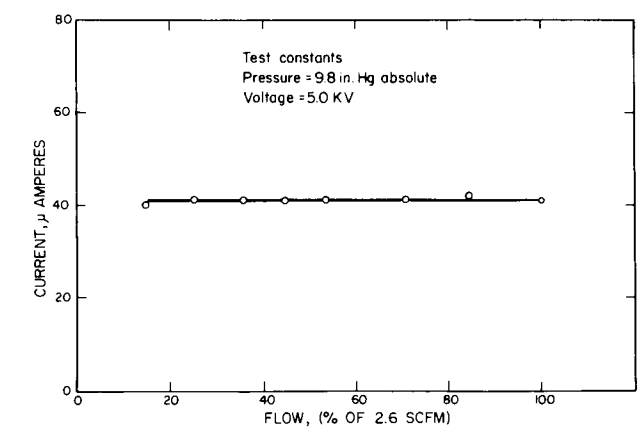


FIG. 7. CURRENT VS. PRESSURE FOR CONSTANT FLOW AND VOLTAGE

FIG. 8. CURRENT VS. FLOW FOR CONSTANT PRESSURE AND VOLTAGE

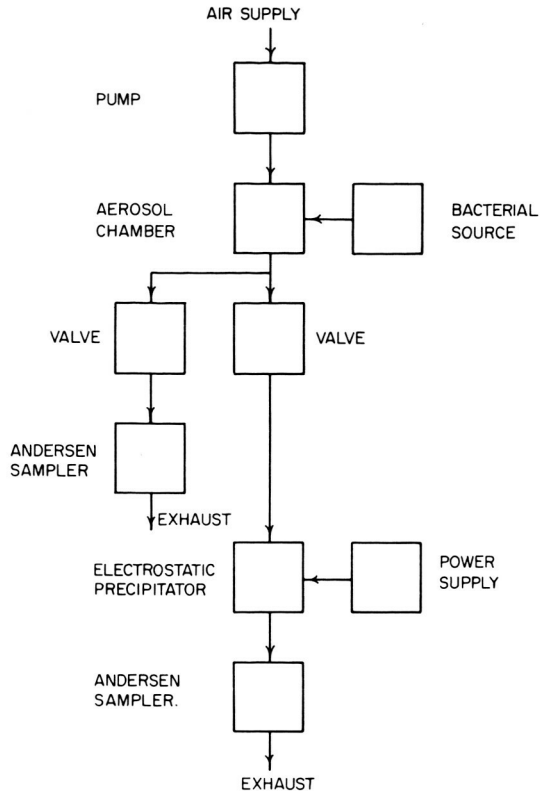


FIG. 9. BLOCK DIAGRAM OF TEST FOR COLLECTION EFFICIENCY OF THE ELECTROSTATIC PRECIPITATOR AT ATMOSPHERIC PRESSURES

Air was drawn from the aerosol chamber through flow meters in two parallel streams. The volume of each was controlled through a calibrated critical flow valve (cf. Fig. 9). One stream passed through the precipitator and then the Andersen sampler. The Andersen here was used to establish the number of organisms not collected by the precipitator. The other stream passed directly through an Andersen sampler.

Andersen sampler colony counts were made by collecting the samples on petri dishes containing tryptic soy agar (Difco) and incubating at 37 C for 24 hours. This medium enhanced the production of the characteristic red color of *S. marcescens*.

The inside of the outer tube was then coated with either high vacuum grease (Dow Corning) or with glycerin to provide for microbial adhesion to the walls of the precipitator. Occasionally tubes were used with no coating on the surface.

The precipitator tubes were autoclaved and then placed in the nylon holder, shown surrounding the glass tube (cf. Fig. 3), using sterile techniques. After the standard run of 2 minutes the tubes were removed from the holder and closed with sterile rubber stoppers. To recover *S. marcescens* the aluminum precipitator tubes were rinsed three times with 100 ml of sterile buffered water and finally rinsed with 5% Tween 80 (Atlas Powder Co., Wilmington, Del.) or with 0.05% Triton X-100 (Millipore Filter Corp., Boston, Mass.).

The effluent was drawn through an HA-type Millipore filter (Millipore Filter Corp.) and incubated on tryptic soy agar at 37 C for 24 hours.

For recovery, about 50 ml of sterile tryptic soy agar were aseptically placed in autoclaved glass precipitator tubes; these were then rolled on an electric tumbler until the agar jelled. These were incubated at 37 C for 24 hours to check for contamination. If the tubes were found to be sterile they were then exposed in the test apparatus to determine efficiency, and again incubated at 37 C for 24 hours. The resultant colonies were counted directly through the tubes.

Uncoated sterile glass tubes were also tested in the precipitator. Sterile liquified tryptic soy agar at 45 C was added to the tube after exposure. As with the coated tubes, these uncoated ones were then rolled until the agar solidified, and were incubated

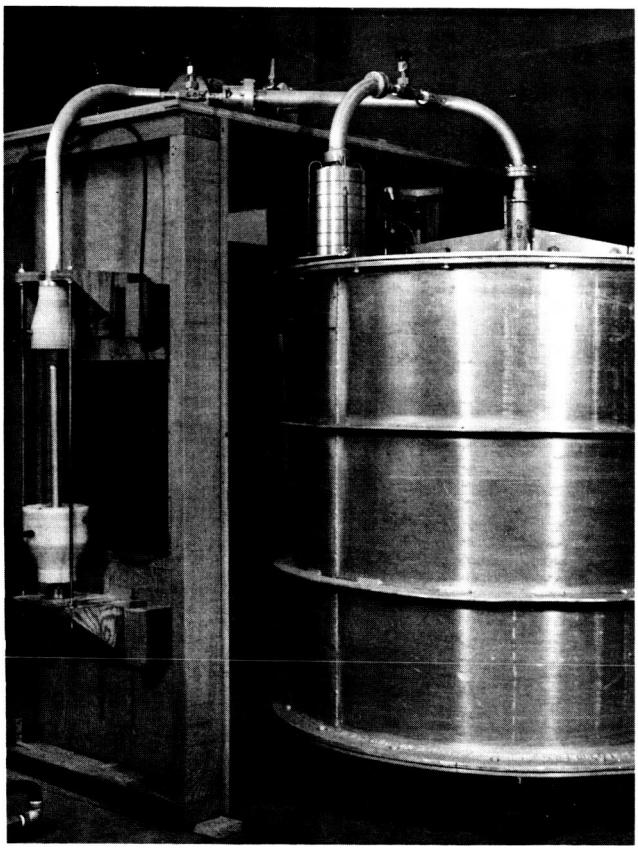


Fig. 10. Aerosol chamber with electrostatic precipitator and Andersen sampler in parallel.

at 37C for 24 hours. The resultant colonies were then counted through the glass tubes.

During the experimental evaluation it became evident that precipitator recovery efficiencies were low. It was surmised that ozone production within the precipitator was a possible cause. As a consequence, experimental studies were initiated to determine the extent of ozone production. A special apparatus was set up to test for the concentration of ozone generated by the precipitator (see diagram, Fig. 11). Air was released through the precipitator at 0.535 scfm for 5 minutes, with the aerosol chamber at 5 psig for a constant pressure air supply.

After passing through the precipitator the air was passed through a modified Smith-Greenberg impinger used as an ozone sampler. The impinger tube contained 50 cc of a 5% potassium iodide solution.

The air was then metered and exhausted. After sampling, the impinger liquid was tested for ozone using Schönbein's method (9). Two milliliters of a 5% starch solution were added to the iodide solution.

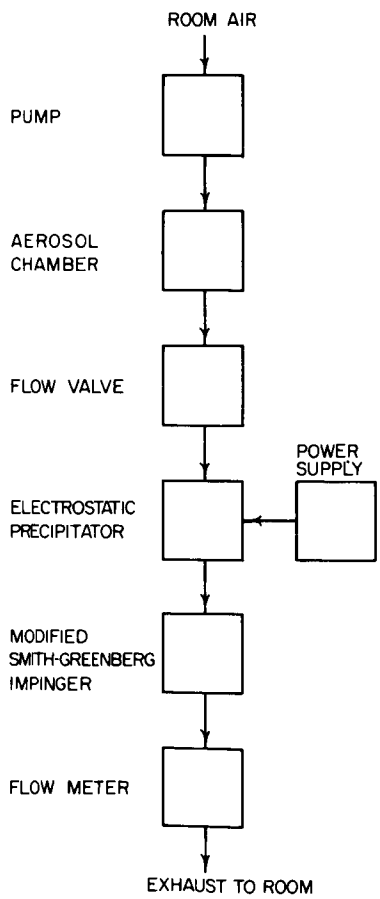


FIG. 11. BLOCK DIAGRAM OF TEST FOR OZONE PRODUCTION

The mixture was then titrated with a 10^{-3} molar solution of sodium thiosulfate.

The concentration of ozone, in milligrams per liter is given by the following:

$$O_3 \text{ conc., ppm} = \frac{(\text{vol. } Na_2S_2O_3, \text{ ml}) \times (2.4 \text{ mg } O_3/\text{ml } Na_2S_2O_3)}{84/\text{liters air}}$$

Two efficiencies from the voltage-related bacterial efficiency tests are important in evaluating the performance of the precipitator (Figs. 12 and 13).

The first, the collection efficiency was computed on the basis of the proportion of *S. marcescens* that was precipitated (or was not precipitated but rendered non-viable by passage through the precipitator) to the total number of organisms entering the precipitator. An effort was made to normalize the data (Fig. 12). This collection efficiency was computed by taking the ratio for each run of *S. marcescens* collected by the Andersen sampler in series behind the precipitator in the flow stream, to the number of colonies of *S. marcescens* collected by the Andersen sampler exposed in the parallel air stream. This ratio was divided by a similar ratio computed from the average of the test runs utilizing the same procedure with the exception that no charge was applied to the precipitator. Under these conditions no ozone was formed. This new ratio subtracted from unity, gives the collection efficiency as a fraction. Collection efficiency increased as a function of voltage applied to the precipitator (Fig. 12). Wide variation in efficiency was evident, however. Negative efficiency resulted from fluctuations in the control runs and from the expression used to define collection efficiency. An alternate explanation might be that we had created life!

The second efficiency, recovery efficiency, (Fig. 13) was computed by taking the ratio of the number of colonies of *S. marcescens* recovered from the precipitator to the number of colonies of *S. marcescens* recovered from the Andersen sampler in the parallel flow stream. This ratio was then divided by the collection efficiency for the same run.

Recovery efficiency appeared to be a function of voltage, but again negative recovery efficiencies were obtained.

In Figure 14 are shown the results of determinations of ozone concentration as a function of current from the precipitator for positive and negative corona discharge. Relatively high concentrations of ozone were produced in the corona.

Discussion

Information obtained with the precipitator at atmospheric pressures may not validly reflect the performance to be expected under the reduced pressures of the upper atmosphere. Tests were conducted primarily to develop analytical procedures with the aerosol chamber and bacteriological techniques with

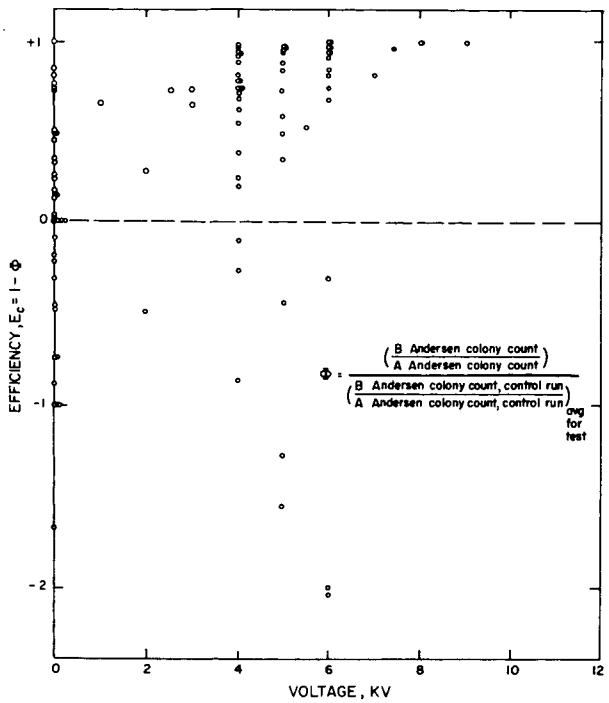


FIG. 12. EFFICIENCY OF COLLECTION VERSUS VOLTAGE BASED ON AN AVERAGE CONTROL VALUE FOR EACH OF SEVENTEEN TESTS OF E =0 WHEN CURRENT, VOLTAGE NOT APPLIED

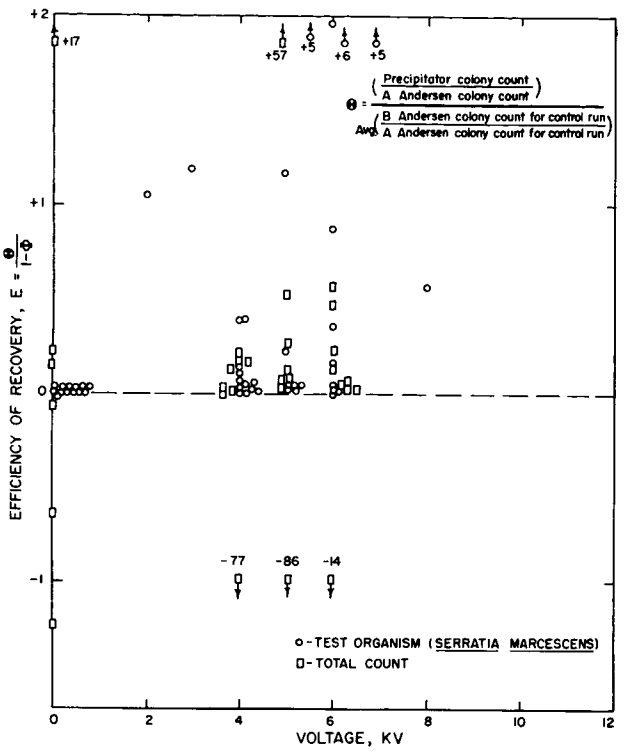


FIG. 13. EFFICIENCY OF RECOVERY VERSUS VOLTAGE

the precipitator tubes prior to conducting low pressure experiments. Superior efficiency possibly can be attained with the precipitator at reduced pressures, because less ozone will be produced. Unfortunately it has not been possible to run the reduced pressure experiments, because of administrative delays in obtaining apparatus. These delays have now been overcome and further testing of the electrostatic precipitator at reduced pressure is in progress.

The principal change in apparatus now implemented is development of a symmetrical flow divider to be used in dividing air flow to the Andersen and electrostatic precipitators. The flow divider used in the currently reported experiments was asymmetrical (Fig. 10). While the quantity of flow in each branch of the divider was equal, some variation in Reynolds number could have caused the unexpected results. Correction of this asymmetry could considerably modify future results.

Conclusions

The physical and electrical properties of the electrostatic precipitator give theoretical promise of an efficient high-altitude bacterial sampler. Unfortunately, low recovery efficiencies have been obtained at atmospheric pressures probably because of the high concentrations of ozone produced.

In the future, ozone concentrations can be reduced by changing the geometry of the discharge electrode and by reducing voltage. It is assumed that low pressure experiments will be essential to fully realize the potential efficiency of the electrostatic precipitator as a high altitude microbial sampler.

Acknowledgment

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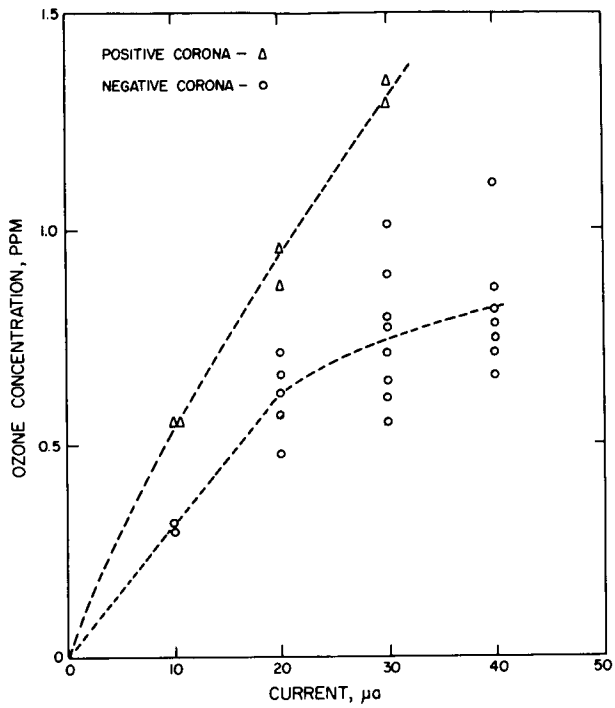

FIG. 14. OZONE CONCENTRATION VS CURRENT FOR POSITIVE AND NEGATIVE CORONA

Discussion

Goetz – How would one operate the Andersen sampler, which works on the impaction principle at low pressure? You will not have any more remotely the acceleration or deceleration that is needed.

Nicholson – We used the Andersen sampler in our lab tests as a comparative sampler to the precipitator. When we go to high altitude simulation we can still use the Andersen sampler as a parallel comparative sampler. It takes a known quantity of air from the aerosol generator at 5 psig, so there is a line coming off the aerosol generator to the Andersen and to the precipitator; it can still be at atmospheric pressure. The other line going into the low pressure end is then drawn down to the low pressure. Now, behind the precipitator one will not be able to put in an Andersen. We either forego that or use some sort of an impinging device.

Goetz – Some time ago we conducted another institute in Europe. We were successful in using a centrifugal device which is pressure independent. Still you can accelerate and produce a dropout independent of pressure conditions.

Nicholson – We have been thinking about this. The problem with centrifugal sampling at high altitude is that one must pump the air through the sampler.

Goetz – No, it's not necessary. One can use a self-propagating device which operates on the impeller principle like the aerosol spectrometer. It is possible to get a considerable air flow under those conditions and still take advantage of the Cunningham correction for the Stokes' fall, which makes these particles drop out much faster while the rate of precipitation remains the same as far as centrifugal acceleration is concerned.

Nicholson – We have been following something similar to this in some preliminary ideas for another sampler which does not involve passing the air through a sampler, or impelling the air in any way. Passing the sampler over the air may be an easier method than pumping air through the sampler.

Brown – I couldn't tell whether you presented the data or not, but is the voltage current relationship dependent upon air velocity in your precipitator, or is it completely independent?

Nicholson – In Figure 8 we have current vs. flow for a constant voltage and a constant pressure. The current is constant, while the change in flow, hence velocity, is between 20 and 100% of 2.6 scfm.

Soffen – I'd like to know what your thoughts are on changes of humidity as a function of the ability of the electrostatic principle to work, especially at high altitudes.

Nicholson – We have no way of controlling the humidity in our test program. The air passing from 5 psig to the equivalent pressure at 100,000 ft is certainly not going to be in equilibrium for humidity. At this time we don't have methods for measuring it. It would be nice to control it. We should measure humidity; we should also measure the ultraviolet light that we're producing.

Soffen – I'd like to just follow my question with some positive suggestion. We've been working on electrostatic methods also and we thought that perhaps it would be logical that as humidity decreases one can expect electrostatics to work more effectively. This worked fine down to a point. The quantitative point I don't know yet because we haven't been able to measure it, but the collection was increased as humidity was decreased down to some point, then there was a sharp dropoff, and finally there was no collection. I've asked a number of physicists for an explanation of this and haven't received a good explanation yet.

Question from audience – Is it a change in viability?

Soffen – No. It's a change in the ability to collect simple particles. They are collected by drying to some point, and then as they begin to get severely dehydrated, collection ceases.

Question from audience – What was the pressure at which you...

Soffen – This was performed at atmospheric pressure.

Question from audience – I believe the problem is the surface film of moisture on the particle, the way it conducts, and the way this changes the...

Soffen – Well, that's the explanation that physicists have offered, but I don't quite understand exactly how that works. If you have an idea I'd like to hear it.

Well, for the sake of the others in the audience who haven't heard it, the sample is at atmospheric pressure. We dried particles and increased our collection efficiency somewhat. However, when the material was made very dry our collection was zero. There was an abrupt decrease in efficiency. The explanation that physicists had given me is that the last layer of water is stripped off each of the particles and that the particles can no longer be charged.

Goetz – The particles were organisms?

Soffen – No, in this case they were road dust, the conventional standard Arizona road dust particles with which most of you are familiar. But stripping off the last water layer sounds extremely simple; I don't quite understand the physics of that either.

MacLeod – How are you drying the air?

Soffen – Simply by heat. We raised the temperature, replaced the atmosphere, and then lowered it, and monitored the humidity over the sample.

Goetz – How high do you go?

Soffen – It's how long, not how high. We don't go terribly high, but we do this for a long period of time and connect it to a vacuum, then return it with dry nitrogen.

Phillips – I couldn't read your chart. When you ran the experiment through an Andersen collector on one side, and on the other side through a charged electrostatic precipitator, with a backup Andersen sampler, were you getting any counts at all on the backup Andersen sampler behind the electrostat?

Nicholson – We would vary the voltage on the precipitator when we did this. We tried to correlate it by saying that when we had no charge on the precipitator we had proportional amounts on each Andersen collector, depending upon how the air flow was divided. When we raised the voltage we got a definite correlation, a definite dropdown on the Andersen counts behind the precipitator.

Phillips – But they did not go to zero as if you were killing all the things that were going through?

Nicholson – The problem is we can't recover them. We can't get them out of the tube. Either they...

Phillips – You couldn't get anything out of the tube, but you got a count on the Andersen after going through the tube? Even though there was ozone present? When your current was on your backup Andersens the air was going through the tube. It was building ozone. The ozone was also passing through your Andersen. Right?

Nicholson – Yes.

Phillips – Right. Now, did you get counts from the Andersen under those conditions?

Nicholson – Our figures showed that the numbers were dropping off as we increased the current, which would happen...

Phillips – Which would happen if you were getting collections.

Nicholson – Yes, and would also happen if ozone were affecting the precipitated particles or the ones caught on the Andersen.

Phillips – But under those conditions you were not able to recover any from your tube, itself?

Nicholson – Very uneven counts. Houwink (3) in Holland, who tried an electrostatic precipitator, had a water bath in one case which spun down the tube. He collected his particles on this water bath; in another case he tried agar-coated tubes. He had good efficiencies, and he was able to recover them. We assume from this that the effect of ozone on a

particle deposited on Andersen plates would not be great because of the protection against ozone afforded by this moisture and this protein.

Greene — I have one comment: You might possibly save yourself a lot of time and trouble by doing the preliminary work with something like fluorescent dusts or dyes, because with the low viability that you are measuring, the overwhelming phenomenon is probably the dehydration of the microorganisms during nebulizing. Until you start disseminating dry particles I don't believe that you're measuring kill by ozone or by impaction, or that you're even measuring efficiency, but that you're measuring an entirely different phenomenon. This has been our experience, and we found that preliminary work on collection efficiency could be done with inanimate particles just as easily. I'd like to ask Dr. Goetz to comment.

Goetz — I wanted to ask the question in connection with Dr. Greene's remark. I have the same feeling. Has the microorganism in your nebulizer been suspended just in an isotonic chloride solution, or has it been in contact with protective colloids?

Nicholson — I don't believe we tried any protective colloids.

Goetz — Skim milk is a famous colloid to make them survive longer. In any case, as Dr. Greene said, one probably has to be afraid to create an artifact which nature may not produce so readily.

Oswald — The question with regard to why we used the vapo nebulizer in preference to dehydrated organisms is an excellent question. We did at first use lyophilized bacteria. We tried a number of different methods for lyophilizing *S. marcescens*: this was, to say the least, quite unsuccessful. When we did use dried samples we found that the samples were collected in the generator rather than in the precipitator or farther downstream in the system. In other words, in the process of producing a collectable dust one would evidently produce unlike charges on the organisms and they would adsorb or stick on any surface that they happened to come to. This was a problem. We then went not to Arizona road dust, but to California dehydrated chicken manure, which we found was subject to the same difficulties. This is why we finally compromised in these preliminary experiments by using wet material from which to generate our aerosol. This aerosol was generated from the original medium in which the organisms were grown, rather than from any special isotonic solutions.

Brown — Do you know where the particles that were in the precipitator picked up their charge? Were they charged when they entered the precipitator or did they acquire their charge in the precipitator?

Nicholson — The charge put on by the discharge in the precipitator is, we assume, a much greater charge than the particles would normally have.

Brown — Do they pick these up by ion bombardment?

Nicholson — Yes. According to theory they do pick up these charges by ion bombardment.

Parameters for Biocolloidal Matter in the Atmosphere

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Abstract

Basic problems of tracing live matter in atmosphere hinge primarily on physical means available for converting this specific form of matter from highly dispersed airborne state into one of much greater compactness at the sensor device, as its ability to produce a signal depends necessarily upon amount of specific matter available to it. An equally basic condition is avoidance of viability interference by process of precipitation.

Parameters guiding this effort can be derived from properties of aerocolloidal matter in general: of the particulates, to be considered more or less *permanent* atmospheric constituents, the *upper* size limit lies for the biosphere at kinetic (Stokes') diameters ($5\mu \leq d \leq 8\mu$), but decreases rapidly with altitude, whereas the *lower* limit for stable particulates of significant frequency appears to be at ($d \sim 0.05\mu$).

This size range embraces, thus, about two decades and includes bacterial cells, spores, virus-like forms, but excludes from permanent suspension pollen, seeds, etc.

Aerocolloidal matter in the average represents but 10^{-7} to 10^{-8} of suspending gas mass and amounts to a numerical concentration of 10^6 to $10^{10}/m^3$ of which the biocolloidal components can be assumed to be only a minute fraction ($\leq 10^{-5}$).

Relation between *kinetic* and *geometric* particle dimensions is discussed, as the former — contrary to the latter — determine duration of atmospheric suspension.

While upper kinetic size limit is determined by high fallout rates, steeply declining frequency for smallest sizes is partly due to increase of *chemical* activity — and thus instability — caused by high surface curvatures (Kelvin relation), and partly due to their *physical* (Brownian) mobility which promotes coalescence.

As most important for preservation of viability, interaction of the particulates with potentially reactive molecular traces in their gaseous environment is discussed, especially under photoactivating conditions.

A similar factor concerns viability interference by contact within close-packed sample precipitates and becomes of major significance whenever coexistent particulates are carriers of photochemical reaction products (such as aldehydes, ozonates, etc.) which are likely to produce toxic effects.

Constitution of natural aerocolloids — particularly regarding their organo-soluble and thermally metastable components — is discussed and illustrated. Author

I want to discuss the subject of the biocolloidal matter in the atmosphere predominantly in the perspective of colloid physics and chemistry, rather than from the microbiological or meteorological viewpoint. My interest in this subject has resulted from the experiences gained by efforts to learn the true constitution of airborne particulates during the last 15 years, i.e., aerocolloidal matter in general, inclusive of its biocolloidal components.

When in 1948 we developed for the first time in this country the controlled production of the now well-known membrane filters and their application to quantitative collection and preservation, specifically of biocolloidal matter from aqueous and particularly from gaseous suspensions (8, 9), we soon became aware of the basic handicaps inherent in its separation from atmospheric environment. In other words, the well-known Heisenberg principle proves itself also here, inasmuch as the *smaller* the system one submits to observation, the more likely it is to be denatured by this process and converted into an

artifact. As a trivial example: A bacterium subjected to high power optical microscopy will soon lose its viability because of the irradiation impact required for its detailed observation — an effect which is by no means restricted to live particles. If, on the other hand, one exposes a very large body, such as the moon, to detailed study, it will not be affected, since the observing system is in this case minute compared to the object. [1]

One of our major targets was to study the principal applicability of these new membranes to the separation and concentration of airborne bacteria. It soon became evident — as to many other investigators since (10) — that particularly vegetative forms are prone to lose viability after a short time of retention on the filters, apparently as a result of the air motion relative to the cell surface. The reason for this appears to be the following: Airborne particulates are virtually at rest with respect to their gaseous environment, hence, the boundary layer at their surface is in a dynamic equilibrium with regard to the colliding gas molecules. [2]

The generally accepted viewpoint in gas kinetics assumes the thickness of this shielding boundary layer around a particle to equal the mean free path of the environmental gas molecules ($\leq 10^{-5}$ cm = 0.1 μ) (4,5). In a "resting" environment the vast majority of the reversible collisions involve the same gas molecules, as their exchange by diffusion processes is slow. If, however, the particle is forced to move relative to this molecular environment, the boundary layer will be disturbed by the shear action of the airflow. Such is the case when the cell is retained on a filter screen and simple estimates indicate that the airflow velocities to which the cell surface may thus be exposed represent appreciable fractions of sonic velocity. The disturbance of this boundary layer is quite likely to upset sooner or later the osmotic equilibria (H_2O, CO_2, O_2, etc.) in the cell.

This type of difficulty has only been mentioned to illustrate the basic handicaps involved in the separation of biocolloidal matter from the airborne state. There exist several methods which minimize this viability interference, such as thermal precipitation or impaction on nutrient agar surfaces (2,32), or in isotonic liquids with subsequent separation therefrom. The least harmful process appears to be the centrifugal deposition by the aerosol spectrometer (20) on a nutrient carrying surface in a resting boundary layer. Hereby changes of pressure and humidity can be avoided even during extended sampling periods, as well as interfering contacts with potentially toxic particulates which coexist in the deposit.

With regard to these problems, relatively simple means may be mentioned which can shield biocolloidal matter against this type of interaction, such as coatings by protective colloids (e.g., casein), and it appears an open question whether the survival of prolonged atmospheric suspension by vegetative organisms may not be due to this sort of surface protection (cf.).

The problem of viability preservation may warrant a brief discussion of the physical and chemical influences to which particulates are exposed in the airborne state (6,12,22). First, one has to discriminate between particle suspensions of *temporary* and virtually *permanent* character — a distinction that depends to some degree upon the rates of atmospheric regeneration, as determined by the locally prevailing micrometeorological conditions. For most purposes one can consider particulate matter of the larger size classes as *temporarily* airborne, since the fallout rate limits their suspension to a few hours. This does not exclude the extremely rare occurrence of much larger particles ($\leq 10^{-2}$ cm) (C. E. Junge, personal communication). In terms of kinetic (Stokes') diameters the border line can be placed between 5 and 8 μ, i.e., individual particle masses of 10^{-9} to 10^{-10} g. From a microbiological point of view this size limit should render the extended suspension of seeds, pollen, and alike highly improbable, contrary to that of bacteria, virus, spores — even of yeasts and certain fungi.

In this connection I want to emphasize the often inadequate *geometrical* definition of particle "size," as it is based on the frequently invalid assumption of a *spherical* particle shape and substances of *unit density*. Only if this condition is realized, the *geometrical* diameters — as resulting from micro-optical determination — coincide with the *kinetic* sizes which determine the fallout rate according to the Stokes'-Cunningham-Knudsen relation (5). In reality, substantial deviations from unit density or spherical shape occur frequently and may particularly be expected for microbiological particulates (24). The application of the geometrical concept can thus cause serious misjudgment of the kinetic behavior of such particles. For instance, a 1-μ lead sphere will act [because of its high density (11.4)] like a 3.4-μ ($= \sqrt{11.4}$) water droplet, i.e., it will fall 11.4 times faster than predicted from its geometry. On the other hand, an 1-μ air bubble with a membrane thickness of 0.1-μ unit density would fall [because of its effective mass/volume ratio of (0.49)] at the rate of a solid sphere of 0.7-μ ($= \sqrt{0.49}$) diameter, i.e., about half as fast as anticipated from its geometrical size. If the bubble

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[1] It may be worthy of note that the mass of the human observer (ca. 7×10^4 g) about centers a log-scale between the moon (ca. 3×10^{25} g) and the lower limit of an aerosol particle (ca. 10^{-16} g) — the ratio being thus about 10^{20} in both directions.

[2] Seen in the perspective of molecular kinetics the situation is as follows: At ground level each cm^2 area experiences every second about 2×10^{23} collisions by the surrounding gas molecules, hence, a bacterial cell of e.g. 1.5 μ diameter and thus 2.5×10^{-8} cm^2 exposed surface receives about 5×10^{15} collisions/second — just about the number of seconds in 200 million years — still impressive, if reduced by a factor of 10^2 under stratospheric pressure conditions.

has the size of 10 μ and the same (0.1 μ) membrane thickness, its effective density (ca. 0.02) would cause it to fall like a solid sphere of 1.4 μ and the duration of its air-suspended state would be about 50-fold longer than predicted from its geometrical dimensions.

Even more critical is the size definition of non-spherical shapes as may be illustrated by the following example: A square plate of unit density with the dimensions (5 x 5 x 0.5 μ) has the mass of a 2.9- μ solid sphere, but the cross section of a 5.6- μ particle. It would thus be expected to fall at a lesser speed than a spherical particle of the same mass, i.e., be equivalent to a kinetic diameter of about 2 μ ($=2.9/\sqrt{2}$) . The microscopic indication for this particle would have been 5 μ , while its kinetic size is close to 2 μ , and its fallout rate – and thereby probable existence in the airborne state – would have been underestimated by a factor 5-6 [1].

As already stated, the upper kinetic size limit of 5 to 8 μ for the quasi-permanently airborne particulates depends on the locally prevailing vertical convection pattern which must compensate the fallout rate. The latter also depends on the pressure, since the rate increases – particularly for the smaller sizes – with altitude, i.e., with the mean free path of the gas molecules. Accordingly, a rapid decrease of the total particle number and in particular of the upper size limit occurs for the higher strata of the troposphere.

With regard to the *lower* particle size limit (not obviously significant in the microbiological perspective) there exist still considerable differences in the interpretation of the available data. It could be expected that their sizes reach into those of the individual gas molecules (10^{-8} cm), but present experiences indicate that under normal conditions the lower limit lies at kinetic diameters of about 0.15 μ , because the frequency of physically definable particulates (impactor, centrifuge, precipitators) in natural, as well as polluted air masses declines (at least in the biosphere) rapidly below this size (16, 17). This evidence appears to be contradicted by nuclei counts, i.e., of condensation centers under varying degrees of H_2O supersaturation. These findings frequently indicate high frequencies of much lesser sizes far into the 10^{-7} cm range (22, 23). The explanation for this discrepancy may be that instantaneous supersaturation in the counter effects a temporary fixation of statistical molecular agglomerates which, without condensation, would exist but for a very brief time interval, hence, could not be classified as "stable" individual particulates.

There are two principal reasons for the virtual absence of stable particulates in the two decades (5×10^{-8} -5×10^{-6} cm) above the molecular sizes: The rapidly increasing mobility of the smaller particles which promotes coagulation to larger units (26,30,33) and the instability of extremely curved surfaces in accordance with the Kelvin relation (12,28). The latter is represented by the diagram (Fig. 1) (18) and illustrates the dependence of the stability in the (geometrical) size range of ($0.1\mu \leq d \leq 10\mu$) in terms of the relative vapor pressure increase (ΔP) over that of a plane surface, under the assumption that ΔP represents also the degree of chemical activity. The curves (A - G) differ for the values of the product [Φ = molecular weight (M) x surface tension (σ)] which characterizes the particle substance with regard to the Kelvin-effect.

The extreme values shown represent: (A) ~ mercury and (G) ~ water, whereas (B - F) concern arbitrary organic substances of increasing molecular sizes from (F ~ M = 100) to (B ~ M = 500) for (σ = 30 dyn/cm).

The significance of this relation becomes apparent by comparing the difference between the (ΔP)-values for water ~ (G), and, e.g., one of the heavier glycols or aldehydes (M ~ 200 ~ E): At (d = 1μ) the (ΔP) -values are less than 1% for both (G) and (E), at (d = 0.3μ) the vapor pressure increase for H_2O amounts to 0.7%, but it is 3.2% for (E), i.e., 4 to 5 times larger for the hydrocarbon which reaches at (d = 0.1μ) an increase of 10.5%, etc. Obviously (ΔP) grows for the same (d) steeply with (M) and thereby indicates the instability of the smaller size range ($d \leq 0.2\mu$) for most organic materials of higher molecular weight. (ΔP) Can thus serve as an inverse measure of the particle stability (or that of a surface layer defined by (Φ).

Atmospheric aerosols of predominantly organic constitution, particularly the smog particulates, show a pattern of size distribution and metastability which meets quite well the postulates implied by the Kelvin law (cf.).

Because of this general, though highly complex type of mutual interaction, the frequency-size distribution of the particulates over the aerocolloidal range ($0.15\mu \leq d \leq 5\mu$) follows in a qualitative sense a closely similar pattern which is quite independent of region and particulate origin. There occurs in general a steep maximum at a smaller size

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[1] The derivation of the kinetic diameters in this range from the light scattering patterns (Mie-Rayleigh) involves no lesser complexities, since the optical properties of the particle substance, as well as the shape enter as influential parameters (29). The application of such methods – if critically applied – can be, nevertheless, most useful, since they are the only ones which do not require the precipitation of the particulates prior to evaluation of their sizes.

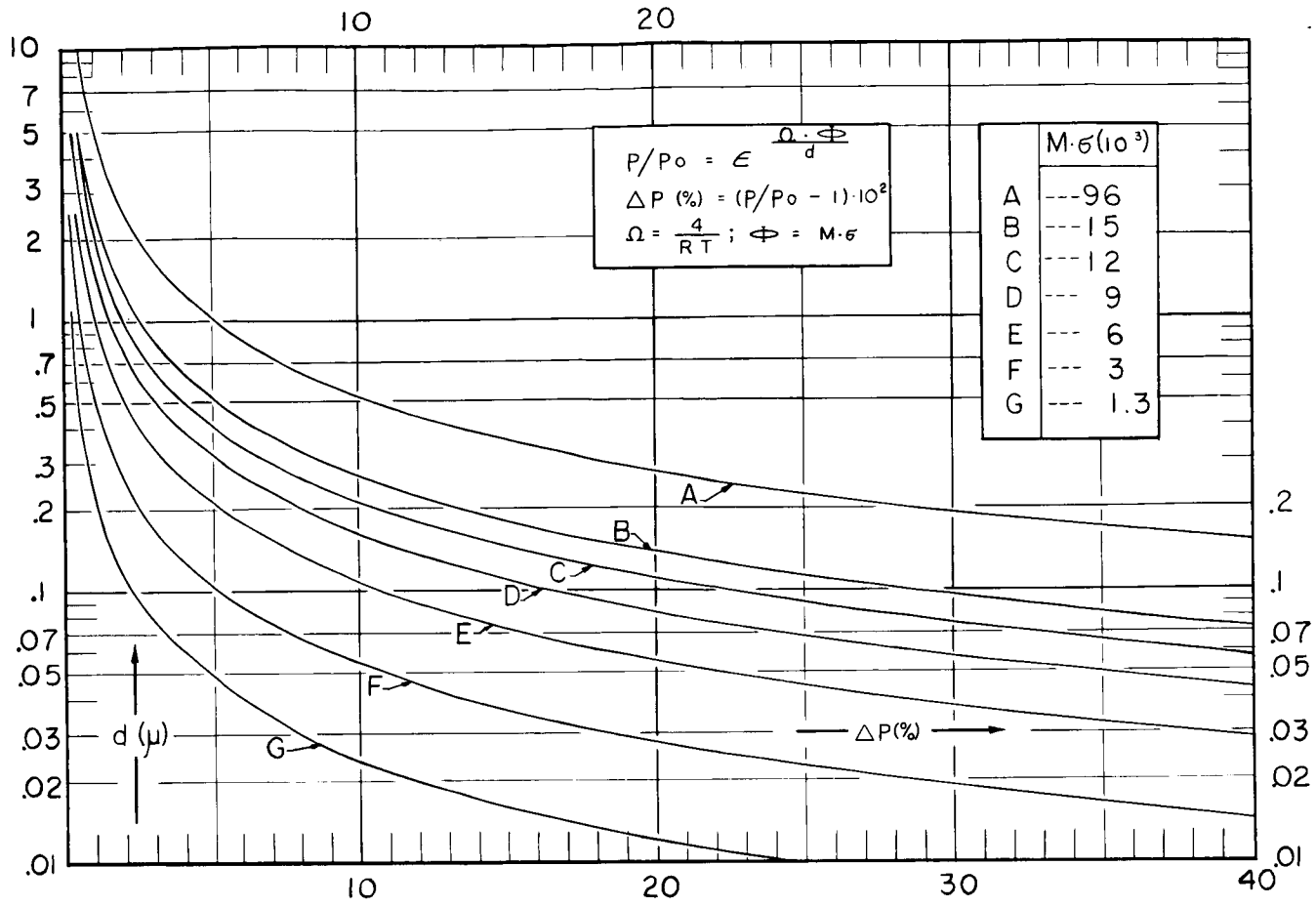

Fig. 1. Kelvin-Relation showing relative increase of vapor pressure (ΔP) with decreasing particle diameter (d) for variety of substances (A-G), varying by molecular weight (M) and surface tension (σ); (A ~ Hg; G ~ H_2O).

($0.2\mu \leq d \leq 0.4\mu$) which is followed by a decline, inversely proportional to the third or fourth power of the diameter ($\sim d^{-3}$-d^{-4}) which indicates a constant or gradually decreasing mass fraction for increasing particle sizes (16,22) (C.E. Junge, personal communication).

For the kinetic pattern of particle interaction these relations are quite significant, for they postulate that the same air volume contains about 1,500 particulates in the 0.2 to 0.3 μ range for each single particle of 2 to 3 μ size – also that the smaller size class does represent a 15 times larger particle surface area, and 10 times the mobility of the larger size interval. This should result in an 150-fold larger probability for mutual interaction either with other particles by collisions, or with reactive gaseous traces in their immediate environment by various types of bonding through adsorption processes.

The inter-particle collisions (if irreversible) cause particle growth, thereby lesser mobility, as well as increased particle distance, and thus a rapid decline of further growth by coalescence while the relatively rare particles which reach the size range of significant gravitational fallout are removed from the system. The result is a steady-state configuration which depends on the rate by which new particles in the smaller size ranges are supplied. Although the total mass of permanently suspended aerocolloids in the biosphere can vary over a wide range due to local conditions of particle origin, washout by rainfall, and vertical convection, its average order of magnitude over larger areas remains about the same and represents (at 30 - 300 $\mu g/m^3$), i.e., but a minute mass fraction (10^{-7} - 10^{-8}) of the supporting gaseous matter.

The corresponding numerical concentration range (10^6 - $10^9/m^3$) suggests, because of the peculiar size distribution, that the combined particle surface area present in the biosphere (except in the arctic regions) equals by order of magnitude that of the earth's surface (15).

These, as such, trivial considerations indicate the inadequacy of defining the aerocolloid concen-

tration by mass, i.e., like dusts. As a matter of fact, a truly representative method for assaying the density and specific nature of aerocolloidal matter appears still to be missing, for it is only partially solved by procedures of light scattering, impaction, or centrifugation.

A most important problem, particularly with regard to viability preservation of biocolloidal matter, concerns the interaction of the particulates with the potentially reactive molecular traces in their gaseous environment. Among such components of the normal atmospheric constituents (N_2, O_2, H_2O), one discriminates between chemically inert types (like CO_2, CO) and those which can react – particularly upon photoactivation – either with other gaseous traces or with the substance of the particle surface. Here the most important representatives are the oxides of nitrogen (NO, N_2O_5, $NO_2 \sim NO_x$).

According to a well-known pattern (21, 25) they effect the catalytic activation of the atmospheric oxygen in the presence of organic traces (i.e., hydrocarbons) which become subject to partial oxidation when excited by photons in the spectral range of about 320 to 420 m μ – as supplied by sun radiation to the lower atmosphere: By this type of reaction, NO_x can produce a large variety of photochemical oxidation products (oxidants) depending upon the nature of the hydrocarbons present. Many so resulting oxidants, particularly those of larger molecular weight ($\geq C_5$), have the tendency to form and accumulate on the surface of coexisting particulates and thereby cause them to grow to many times the original size (13,19). This in turn produces restriction of visibility and may cause synergistically intensified irritation (1,11), i.e., the typical smog reaction. The particulates present during this reaction appear thus to act in analogy to "condensation nuclei" (in aqueous fog formation), as catalytic centers which promote the reaction as such, evident from the fact that the particle growth is within wide limits independent of the concentration of such reaction centers (19). This appears particularly significant for biocolloidal particulates, since it can be expected that photochemical reactions on their surface will affect their viability, as traces of NO_x or active oxygen are known to be present also in unpolluted air (7). Therefore, it appears doubtful whether airborne organisms can survive periods of extended irradiation exposure by the sun.

An equally significant problem concerns the chemical *stability* of aerocolloids: Detailed studies of natural and of synthetically produced aerosols have shown consistently that major fractions of the particulate substance are unstable, as evident from their gradual shrinkage upon additional irradiation or moderate exposure to heat (16,17). This indicates that a large fraction of the aerocolloidal substance represents intermediate oxidation states of organic matter which is gradually converted into more volatile and stable end products (CO_2, H_2O, and probably NH_3OH) (7). Studies of particulates which cause "hazes" in polluted rural, desert, forests, and off-shore maritime air masses have also indicated a varying degree of metastability depending on location, etc., but never its entire absence – at least in the biosphere. They differ in this respect from urban smogs by much lower concentration and the absence of irritant capacity – most likely due to the different nature of the organic reactants originating from the metabolism of vegetative life (31) on the continents and in the oceans (3,27).

The above discussed indications are illustrated in the following: In Figures 2a and 2b are shown the size distributions of two typical marine aerosols, derived from deposits with the aerosol spectrometer. The ordinate (C) indicates the number of particles (in arbitrary units) vs. the geometric diameters of salt particles (d_s) and those for droplets of the saturated solution (d_L), based on the corresponding density ratio ($\rho_L/\rho_s = 0.52$). Figure 2a refers to a morning haze (87% RH) over the ocean, slowly drifting ashore, where all particles should be presented by droplets (d_L-scale). A distinct maximum for ($0.3\mu \leq d_L \leq 0.33\mu$) and a steep decline toward smaller and larger sizes is evident. This decline toward larger sizes is steeper than ($C \sim d^{-3}$), namely, that of a constant mass distribution over the size range, as indicated by the dashed curve.

Figure 2b was taken on a clear day about 40 miles offshore at 55% relative humidity and shows two distinct maxima for ($d_s = 0.21\mu \pm 0.01$) and ($d_L = 0.46\mu \pm 0.01$). The ratio ($d_L/d_s = 0.22 \pm 0.2$) coincides closely with the calculated ratio of the kinetic diameters for identical salt particles in saturated solution or as crystals (i.e., $d_L/d_s = 0.209$). This observation is quite typical for maritime conditions and can be interpreted as the coexistence of a dehydrated and a hydrated fraction of the same salt particulates, either because of its different "age," or because of factors delaying the evaporation of the condensate.

In Figure 3 is shown a similar marine aerosol pattern (obtained with the aerosol spectrometer) in terms of the light scattering intensities (S_d) and the numerical concentrations (C_d) vs. the kinetic diameters for the range ($0.1\mu \leq d \leq 1.4\mu$). ($C_d$) refers to the particle number (cm^{-3}) in the size interval of ($\Delta d = 0.05\mu$). The thermal metastability is determined by the repeated evaluation after exposing the deposit to 65 to 70 C for 12 to 15 hours. The decline of (S_d) and (C_d) for the smaller particles is obvious and follows a general pattern to be expected from the Kelvin relation (s.a.), and thereby indicates the presence of other than mineral components, as pure salt particles should not decompose in this temperature range. The dark-field micrographs of equivalent deposits before *left* and after *right* heating serve as further illustrations of this general phenomenon.

In Figure 4 are shown the corresponding size distributions for aerocolloids of vegetable origin (alfalfa grove) and in Figure 5, those typical of Los Angeles smog. The latter differs in particle number by a factor of about 20 from the former

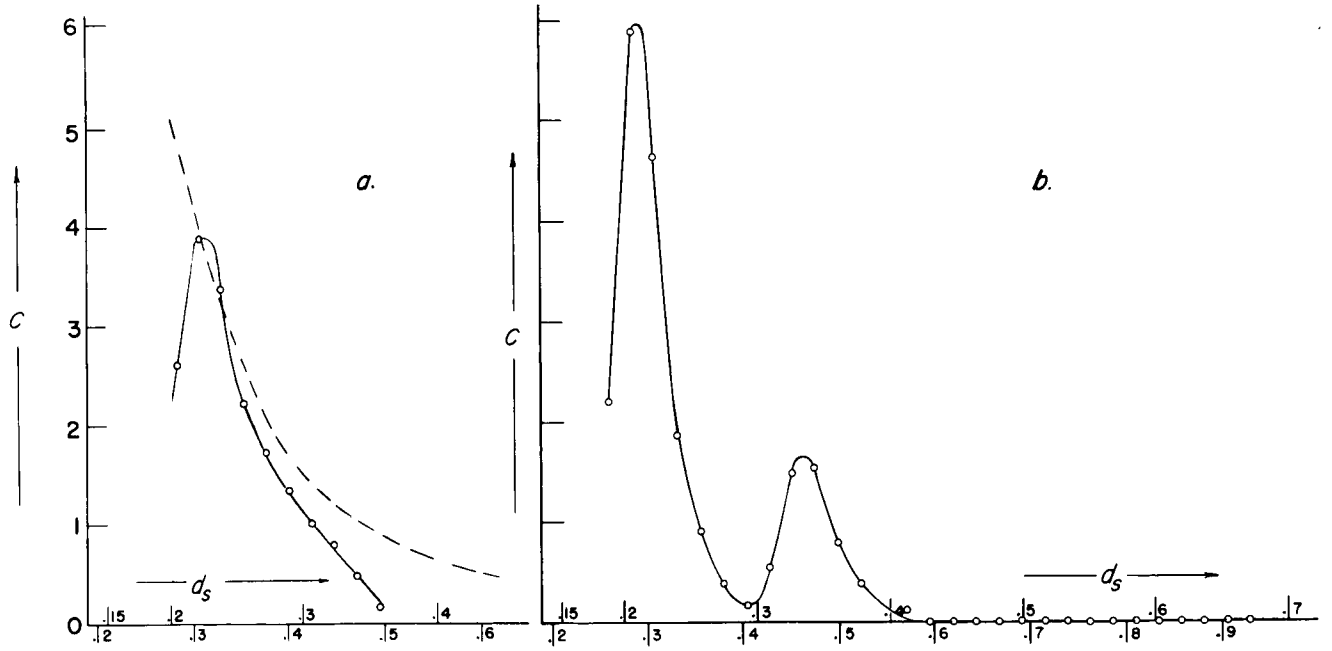

Fig. 2. Size-frequency distributions of marine aerosols: C ~ particle number for size interval ($\Delta d = 0.05\mu$) vs. diameter of salt particles as crystals (d_s) and as saturated solutions (d_L) (16): a) onshore moving haze, RH = 87%; b) air mass 40 miles offshore, RH = 55%.

and also by a much larger metastability for sizes ($d \leq 0.8\,\mu$). The corresponding photomicrographs again illustrate the disappearance of the smaller particles.

From the viewpoint of biocolloidal components, the almost generally observed presence of the metastable, most likely organic components of the particulates appears of considerable interest, while their origin and ultimate fate must remain at present still subject to speculation. Their organic nature can be demonstrated by a rather simple method: By a slit-impactor, designed specifically for this purpose, an aerocolloid deposit is produced on a highly polished chromium surface and then subjected to dark-field microscopy under low angle reflected illumination. When the relative humidity is increased to > 80%, the gradual formation of water droplets around active condensation centers can be visually observed — and upon relative humidity reduction the subsequent evaporation pattern. In Figure 6 this is shown for the same deposit fraction at four different stages for an offshore air sample taken 15 to 20 cm above sea level. The presence of a barrier around each droplet becomes apparent from the absence of circular shapes — also from the fact that the evaporation is slow, extending over several minutes after humidity reduction to 30 to 40%.

The evidence of the organic nature of this barrier is given by its removal with one drop of solvent (chloroform, ether, etc.) applied by a syringe run along a fraction of the particle deposit, and then allowed to evaporate (14). In Figure 7 is shown the resulting pattern in dark- and bright-field reflected illumination, where the *left* section of the (diagonal) impaction deposit had been contacted by the drop *(center)* prior to evaporation, while the *right* section remained untouched.

The effect of this extraction on the dynamics of the condensation process is shown in Figure 8, where the three micrographs on the *left* show the same impaction deposit before chloroform extraction, while the corresponding ones on the *right* show the residue thereafter. The pair (1) is the deposit at low magnification (equivalent to 5 liters), (2, 3) present a deposit section at higher magnification: (2) *during* supersaturation, and (3) the residue *after* subsequent evaporation. Prior to extraction the pattern (2) develops and vanishes slowly and the drops show no tendency to coalescence — closely similar to Figure 6 — whereas the extracted pattern forms almost immediately and coalesces in less than a second. The left (3) shows the unchanged original particulate pattern, whereas the right indicates the formation of a few large centers resulting from the coalescence of the droplets during condensation.

The data available at present for the biosphere indicate that organic surface layers on particulates are quite common; however, nothing is known about the extent to which they influence the viability of organisms. It is thinkable that they may act as an envelope which protects the sensitive enzyme structure on the cell surface against reactive molecular traces of their gaseous environment — similar to the

aforementioned shielding by colloidal matter. A similar function could be the protection of the cells against fatal degrees of dehydration.

Systematic studies (in particular regarding the photochemical reactions of such barriers) would promise to reveal valuable data about viability preservation at higher altitudes, especially for the passage into the stratosphere.

Generally speaking, it appears that the low temperature level in these layers can be expected to be a preservative factor against chemical degradation; on the other hand, the intensity of photoactive radiation may overcome this thermal inertness for the viable systems.

Beyond these factors, the probability of their extended presence in the upper strata can only be judged from their gravitational fallout pattern. This, in turn, depends on the mass-minimum, required for a "live" unit – defined by its ability to "catalytically" convert suitable environmental matter to its own structure.

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Nomenclature

C	Number of particles
C_d	Numerical concentrations
d_s	Diameter of salt particles
d_L	Diameter of droplets
M	Molecular weight
Φ	Molecular weight x surface tension
P	Vapor pressure
σ	Surface tension
S_d	Light scattering intensities

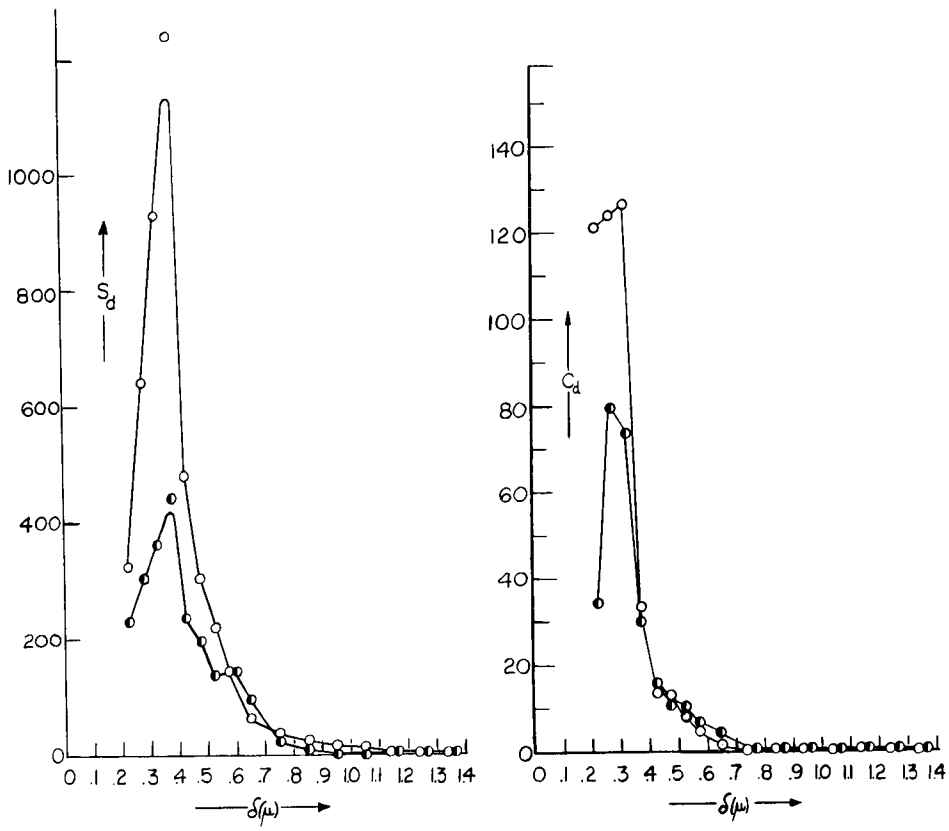

Fig. 3a. Size-frequency distribution of marine aerosols in terms of: light scattering $\left[S_d(d)\right]$ and count analysis $\left[C_d(d)\right]$ from deposits obtained by the aerosol spectrometer.

Unfilled circles prior to, and *partially filled circles*, after storage of deposits for 12-15 hr at 60-70 C. (C_d) indicates the number of particulates per cm^3 for ($\Delta d = 0.05\mu$) (17).

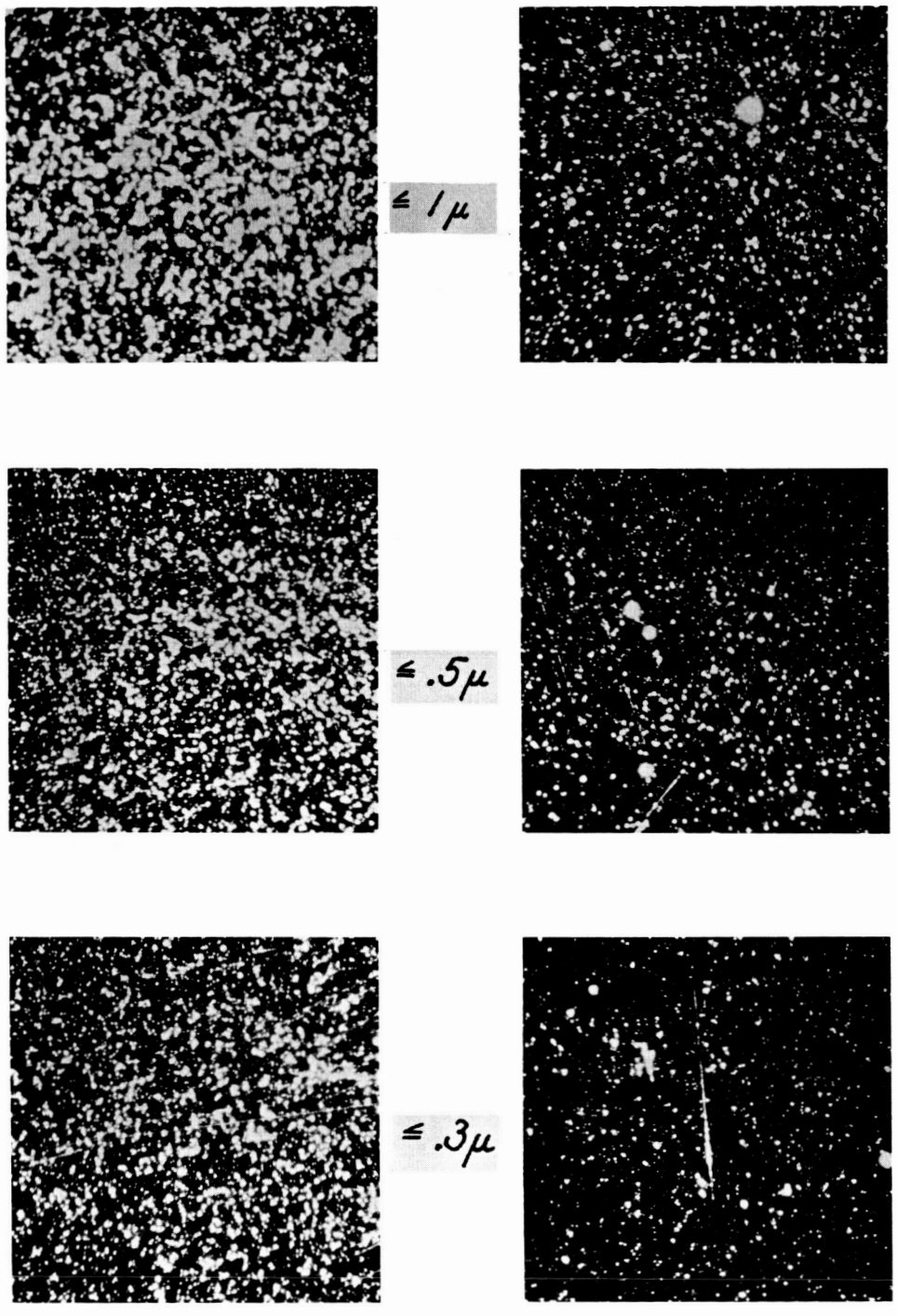

Fig. 3b. Dark-field micrographs of same deposit sections in three different size ranges, before *(left)* and after *(right)* heat exposure.

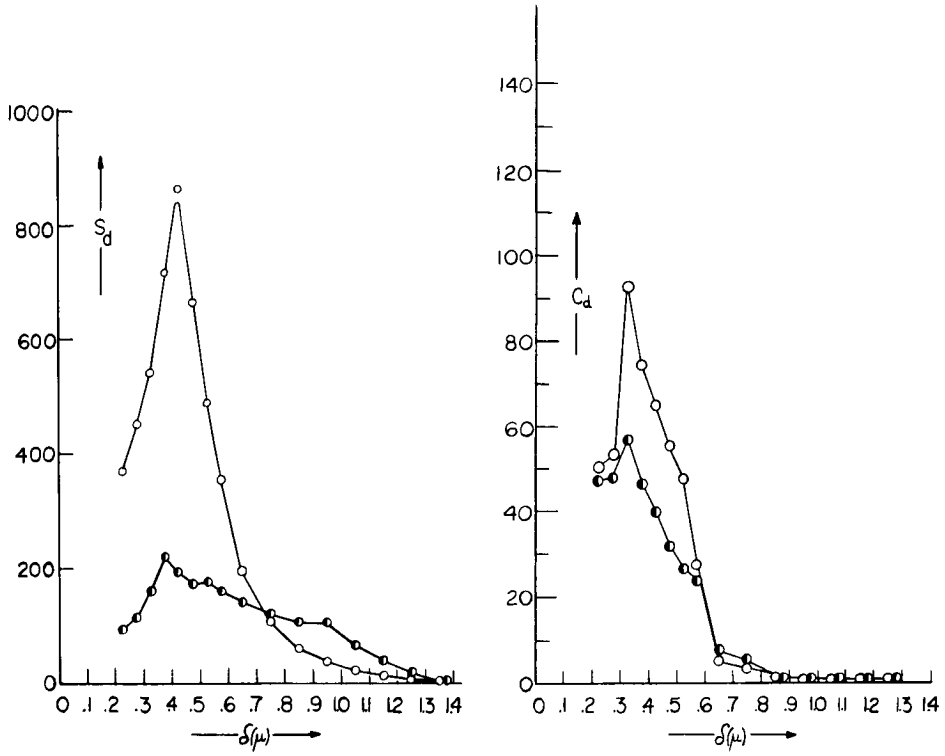

Fig. 4a. Size-frequency distribution of aerosols in dense alfalfa grove (North San Joaquin Valley, Calif.). Codes same as in Figure 3a & b (17).

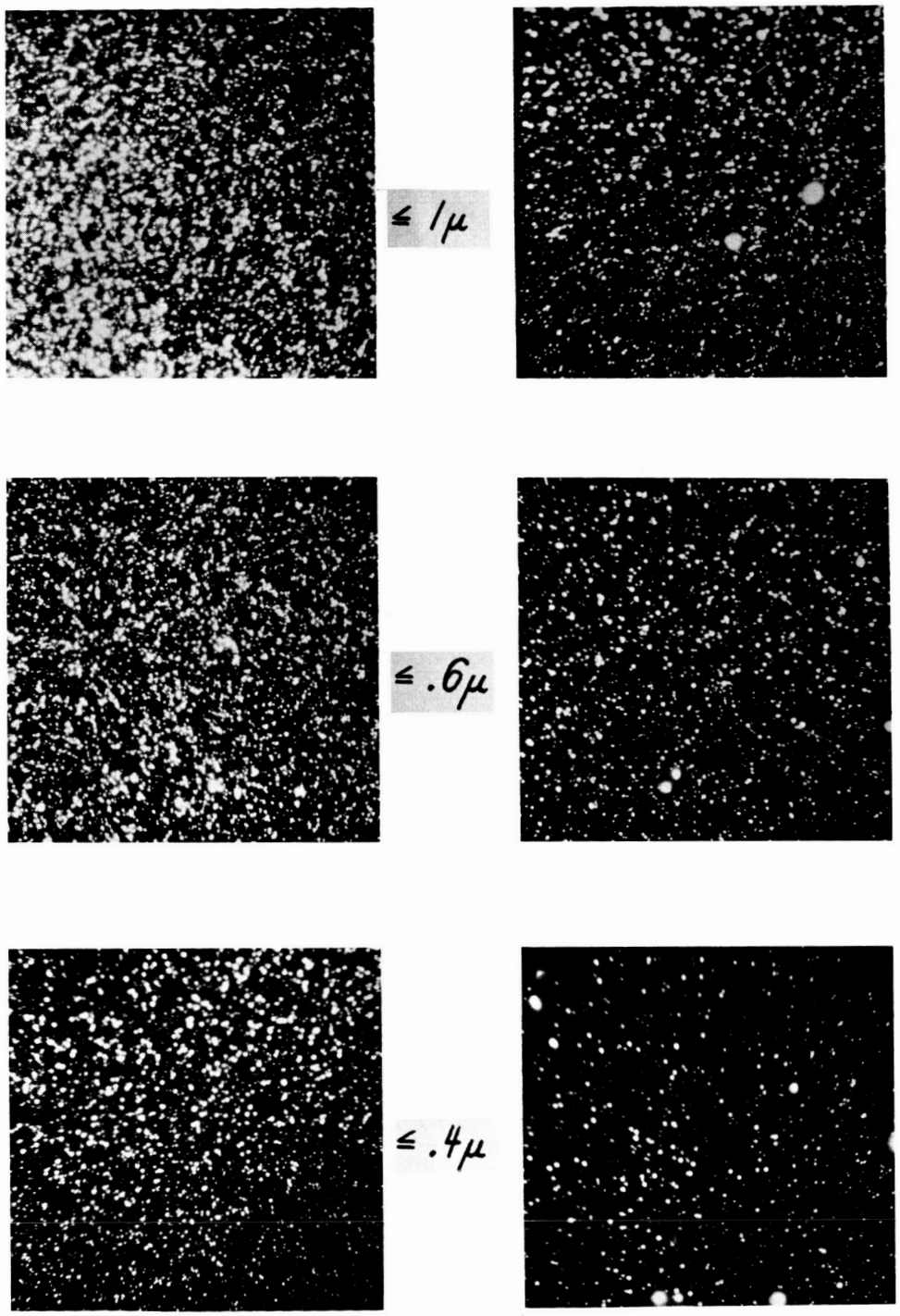

Fig. 4b. See Figs. 4a and 3a & b for explanation.

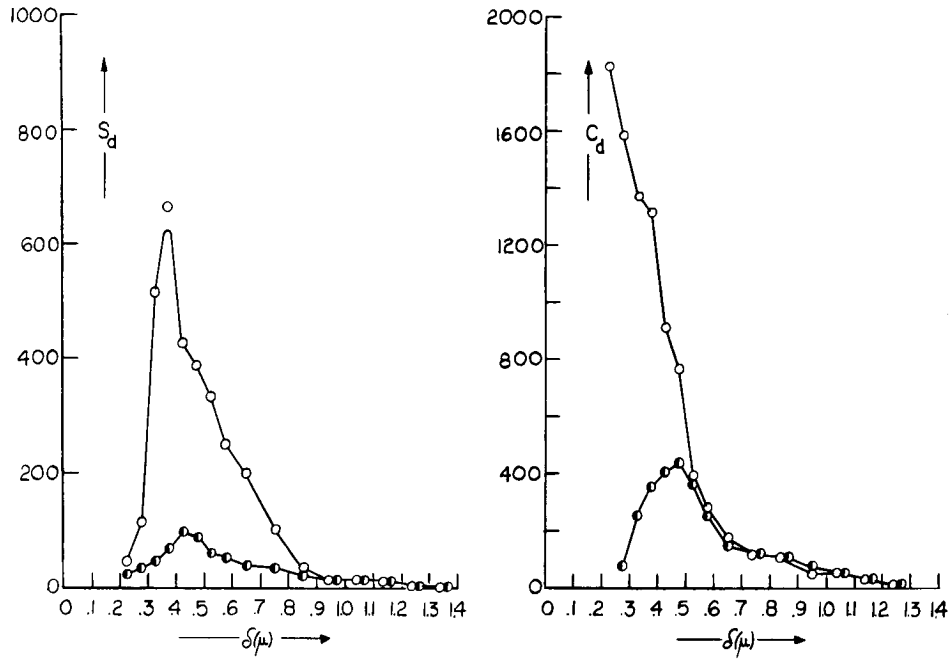

Fig. 5a. Size-frequency distribution of aerosols in Los Angeles smog. Codes same as in Figure 3a & b (17).

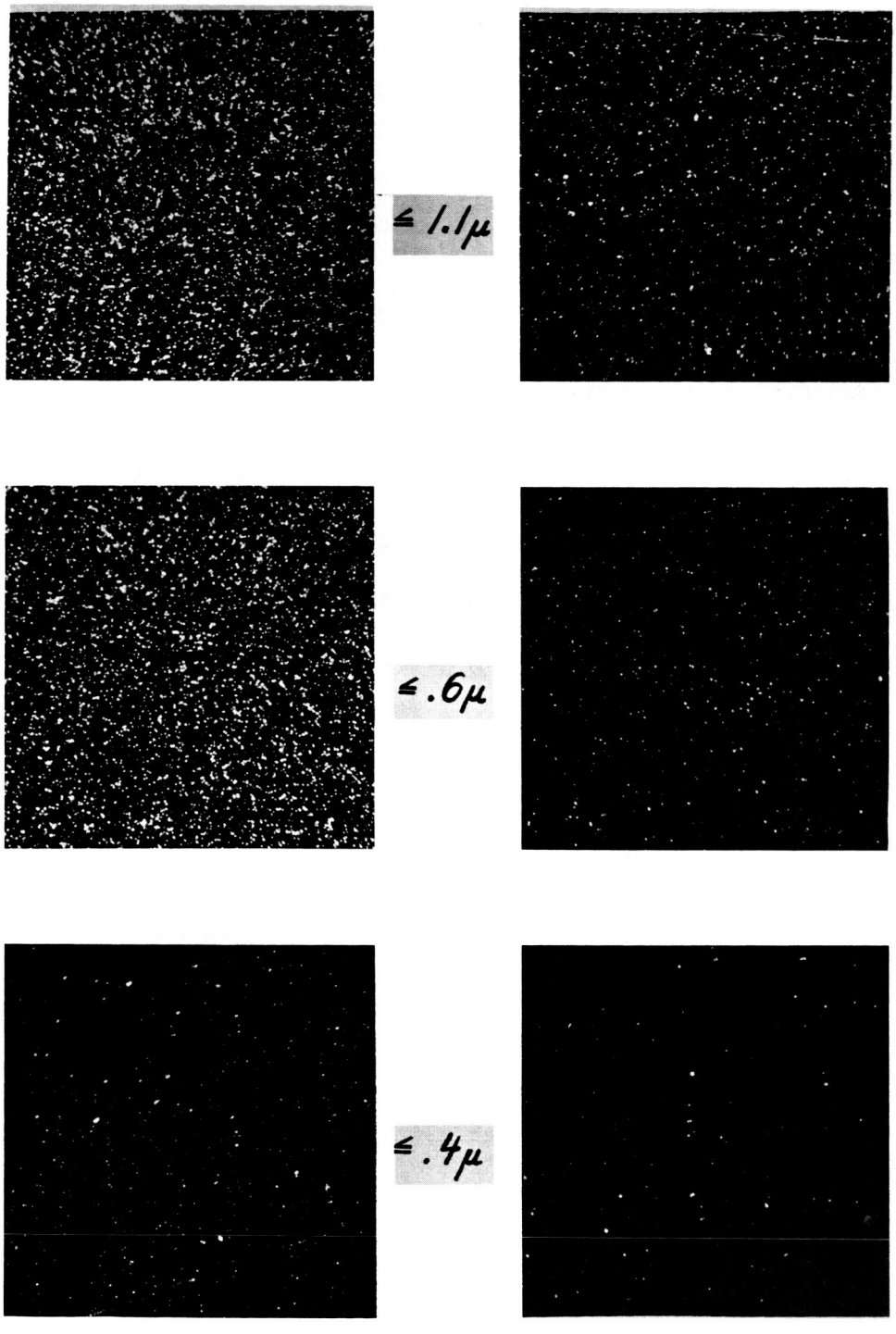

Fig. 5b. See legend on opposite page.

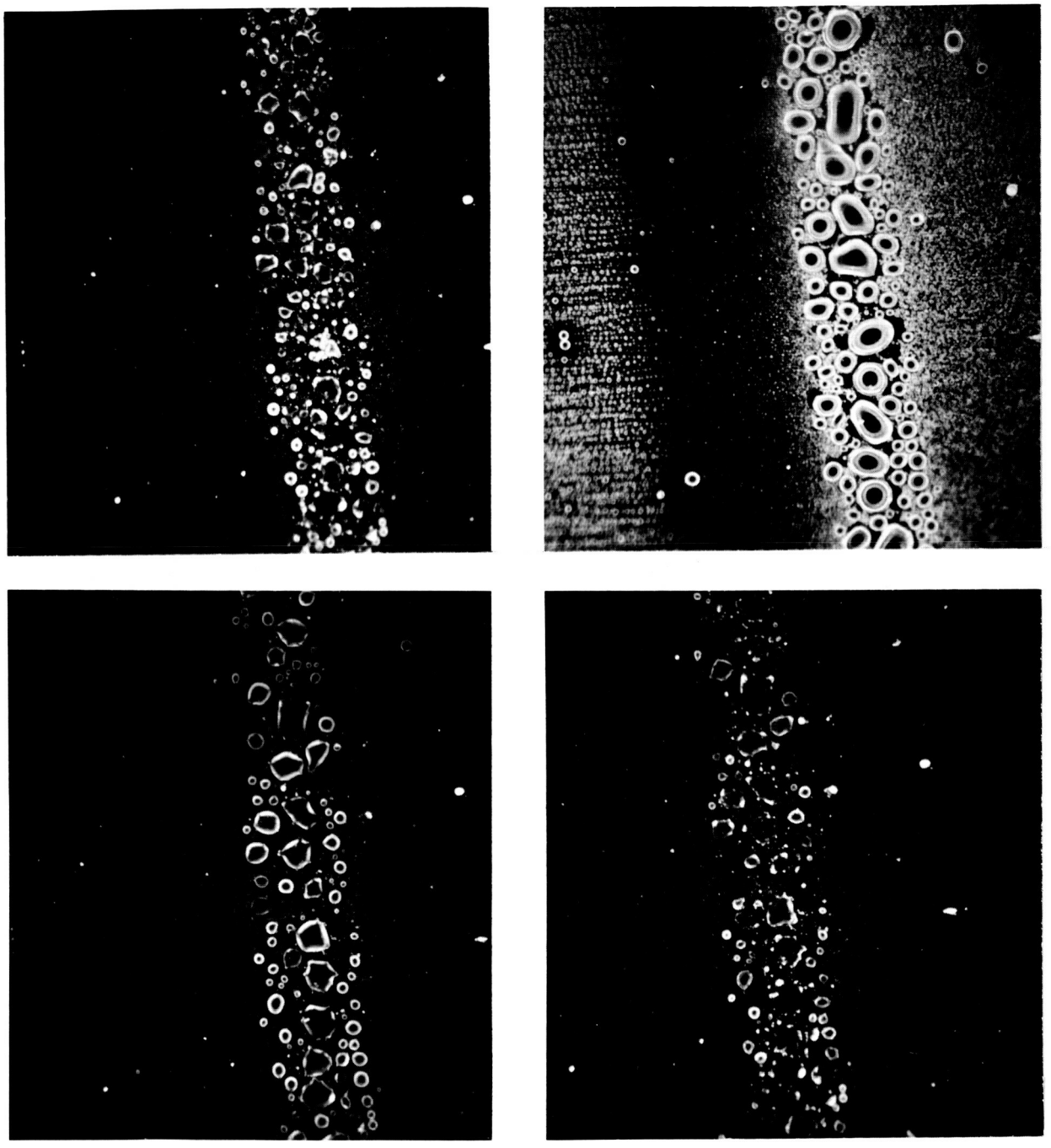

Fig. 6. Dark-field micrographs of condensation patterns on deposits by chromium-foil impactor representing marine aerosols, close to (6 inches) the ocean surface. Upper pair shows same deposit section *before (left)* and *during (right)* condensation; lower, during gradual evaporation *(left)* and about 10 minutes thereafter at low relative humidity *(right)*.

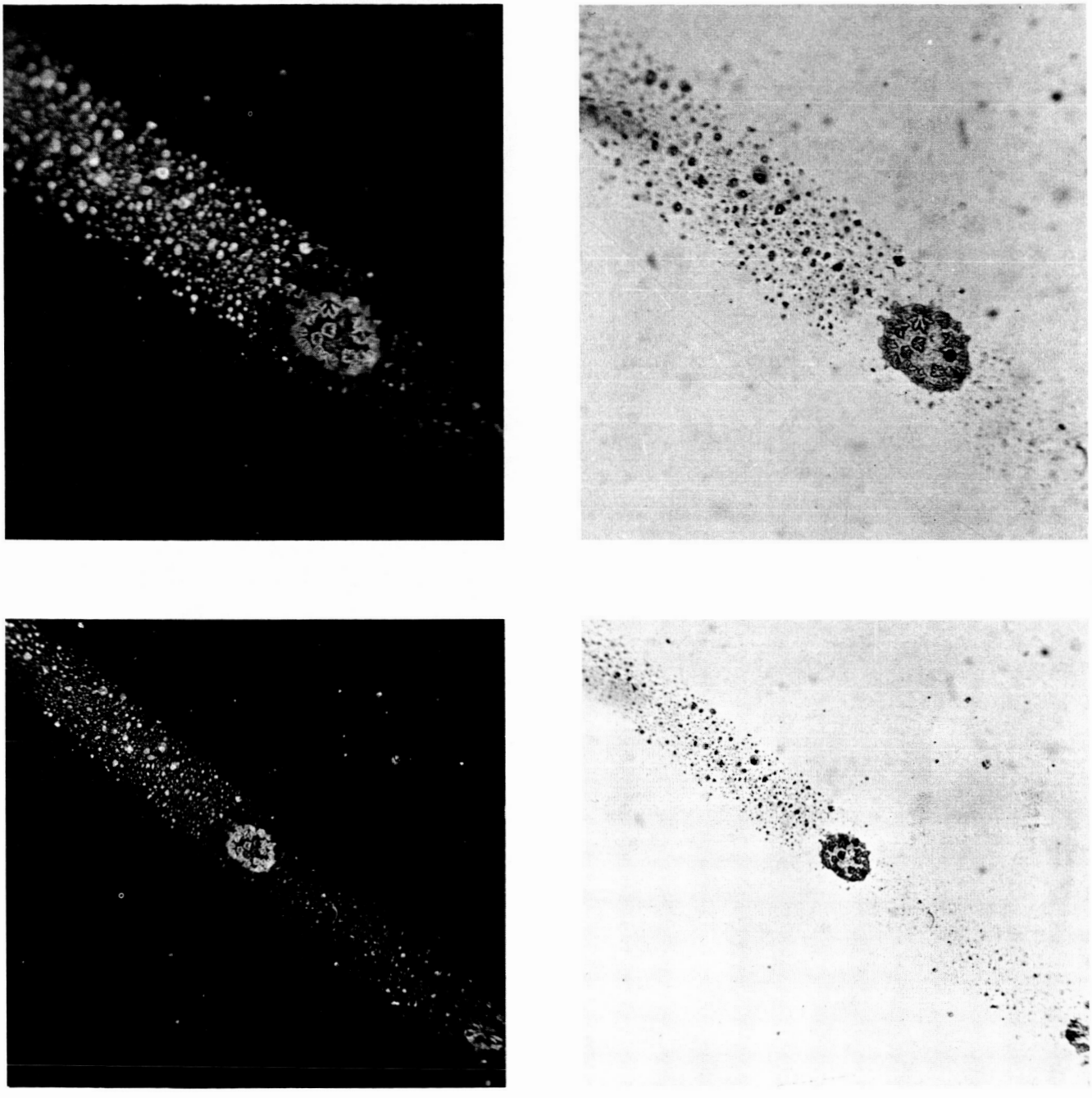

Fig. 7. Micrographs of similar deposits under dark- *(left)*, and bright-field *(right)* reflected illumination after partial contact *(lower right)* with chloroform drop, subsequently evaporated in center. High power *(top)*, low power *(bottom)*.

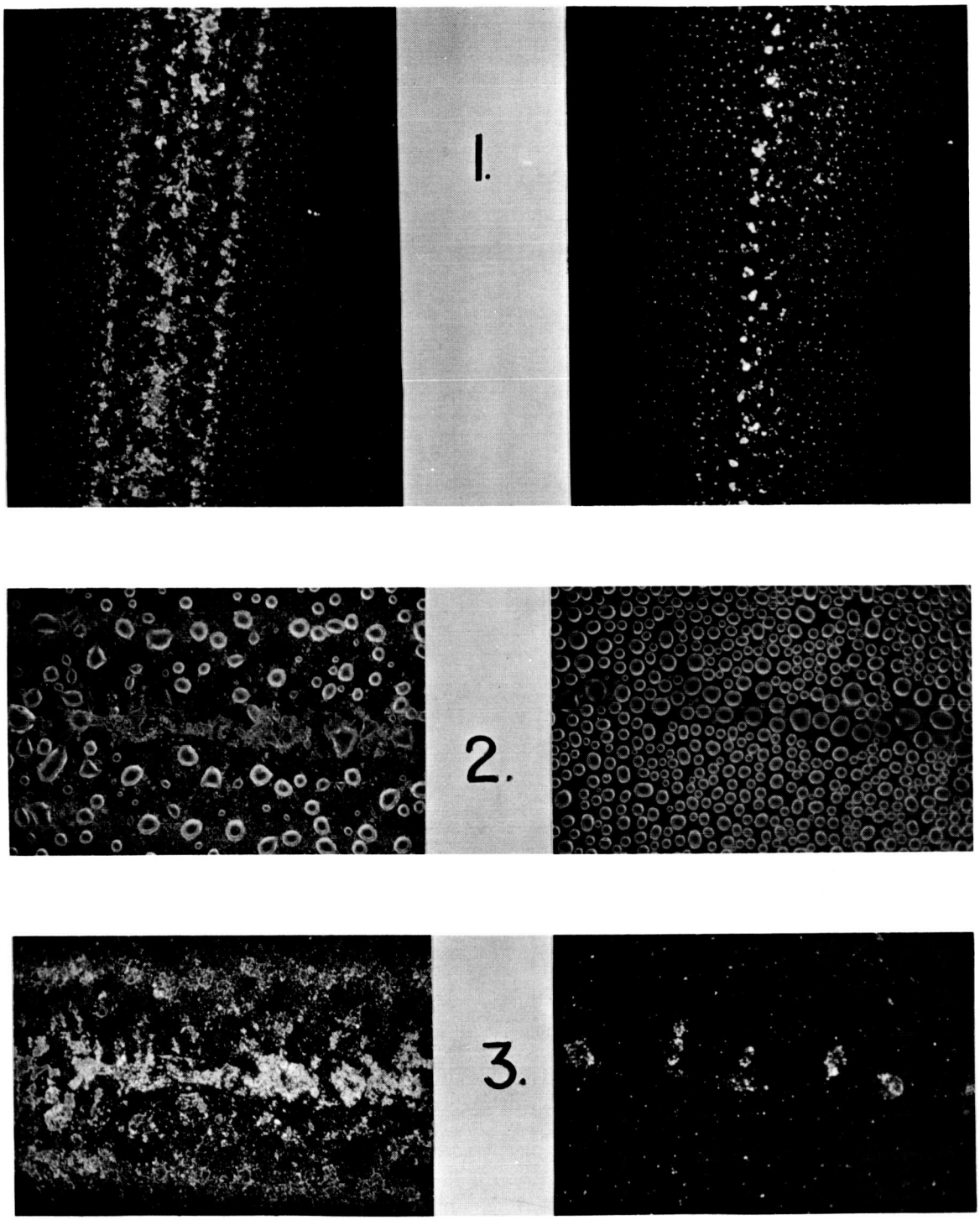

Fig. 8. Dark-field micrographs of condensation patterns prior *(left)*, and after *(right)* chloroform extraction on the same impaction deposit. Upper pair shows deposit at low magnification *before* condensation, the other pairs, sections thereof *during* condensation and residues *thereafter*.

Discussion

Junge — I have two questions: 1) did you also collect such particles in higher layers from aircraft, and 2) was there a similar phenomenon of evaporation of the smaller ones that you showed with the surface samples?

Goetz — The highest altitude we had an opportunity to go to was just below 20,000 ft above the desert of northern Arizona in connection with Dr. Weickmann's cumulus project. We used the same equipment — aerosol spectrometer, impactors, and nuclear counters — on board the airplane, as on the ground tests. Thermal stability at sunrise and instability before sunset were definitely indicated over the Grand Canyon region at 2,000 to 3,000 ft (9-11,000 ft above sea level). The total particle number (which for instance in Yellowstone Park we found between 100-200 cc) dropped at that altitude to about 20 to 30, whereas at desert ground level the number was 50 to 100/cc. The air mass in the Grand Canyon is the only place in this area where we have found almost complete stability even after sun irradiation. These samples, however, will have to be repeated for confirmation.

Junge — If you observed this difference in stability between sunset and sunrise, this would imply that the particles overnight would evaporate a certain part of the material which they accumulated during the day?

Goetz — I believe so. The sun irradiation seems to produce a type of compound which renders the particulates metastable along the same pattern as the smog. In the Yellowstone region, we sampled in the same manner on days in which the sky was completely clouded — even when it was snowing — at about the same time of the day and no particles could be found at all. Similarly, I had a horizontal hole dug about 5 to 6 inches wide into a 5-ft snow wall. Nothing whatsoever could be traced therein; one could thus believe that this effect is some sort of a photo- or thermophoretic phenomenon, due to the intense sunlight.

Benninghoff — Could you explain again, Dr. Goetz, the evolution of the particles over the snow surface. I didn't follow your explanation of the manner in which they were evolved.

Goetz — I can report only what we found, and like to suggest that the effect could also be due to a Stephan phenomenon, that is, a molecular flow of evaporated water from the snow surface, which would prevent the contact of the particles with the surface. It appears most unlikely that the snow itself yields these particles. We recorded the surface temperature of the snow at -15C so that melting was out of the question, while we had extremely bright sunlight. Conceivably we had some sort of a radiometric effect, thermo- or photophoresis, which caused the particles to move away from the source of radiation and to concentrate above the snow surface, entry into which the Stephan effect would prevent.

Benninghoff — I have a second question. Have you had occasion to study the metamorphosis of these atmospheric colloids or aerosols under conditions of heavy cloud cover?

Goetz — In a few cases we have but preliminary evidence: During the mentioned flight tests over Arizona, we penetrated occasionally some clouds where we found a large amount of small, thermally stable particles. Similarly, flying through an accidental rain shower for about 30 seconds, we obtained an amazing quantity of this particle type. At recent tests in about a 40-mph rain blow at sea (Santa Catalina Island channel) our impactor samples also consisted of particles that apparently were virtually unextractable with organic solvents.

Belmont — It might be interesting to try your experiments over a place like the Greenland ice cap during the polar night and during the polar day; first of all, the large extent of ice and snow removes any gluten sources, so there is a fairly clean air mass, and second, to detect any day and night effect.

Goetz — Drs. Weickmann and Fenn did this north of Thule several years ago with the same instrumentation, and found barely any particles. This experiment concerned an air mass which had been confined to the ice cap for about three weeks, and thus was not likely to contain organic trace matter.

Question from audience — This was in summer during daylight?

Goetz — I seem to remember that it was during the midnight sun period.

Danielsen — Do I understand you correctly, or infer correctly that if sunlight were to irradiate these particles when there were water molecules present, there would be a greater tendency for drop coalescence?

Goetz — May I correct this. Let me repeat: we produce such a deposit on a carefully-cleaned chrome foil. Chrome surfaces are chemically neutral and can be brought to a polish which effects a complete dark field in the microscope upon low angle (20^{o}) illumination. First, this chrome foil is rinsed with the same solvent we use later to dissolve and remove any surface impurity. After about 15 seconds impaction, we obtain the pattern on the left side of Figure 8. When we increase relative humidity over the sample while under the microscope, to produce condensation in the particles, we observe first no change, then the gradual growth around most centers until the droplets virtually touch one another. At the contact area they will form a flat surface but very few coalesce. If we cause the particles to shrink by reducing the humidity, they come slowly back to their original form, thus the deposit returns in a few minutes to the first pattern. If we now extract this deposit by running a drop of chloroform or ether over it, the pattern can be completely changed, as we have supposedly removed the organic components with the solvent drop without affecting the mineral constituents. If we then produce condensation on this

residue, we obtain the pattern on the right side of Figure 8: the droplets grow in a fraction of a second, faster than one can push the camera trigger. If the droplets have grown to a certain size and touch another, they coalesce into a few big droplets. Humidity reduction effects rapid dry-off and leaves but a few centers.

The particles apparently collect this protective envelope after prolonged exposure to sunlight. We can produce this effect (smog reaction) synthetically in the laboratory on latex particles (8) by intense irradiation in the presence of NO_x and aromatic or olefinic hydrocarbons which produce by the reaction a substantial mass increase of the latex centers.

Cole — There are both of these, one from water and one from snow. Is the water necessary in this process or will an organic material such as a leaf produce a similar sort of thing?

Goetz — If I understand your question right, is it necessary to sample above a water or a snow surface?

Cole — You understand correctly.

Goetz — No, it is not necessary. You may remember that I showed a slide from an alfalfa grove over 100 miles inland. We obtained similar deposits in the Mojave and above the Navajo Deserts, but to a much lesser degree than over the oceans or within the forests. Our impression is that one needs photons and the metabolism of live matter to produce such aerocolloids. This is apparently a mechanism (F. Went suggested this originally) to volatilize by photo-oxidation the non-fermentable end products of life. If this did not occur, we would probably have to live in miles of wax (18).

Soffen — Dr. Goetz, have you considered trying to assay the volatile material on the thermally-labile substance with a sensitive method like gas chromatograph?

Goetz — The minute mass concentrations represent the basic difficulty in identifying aerocolloid constituents as they represent only 10^{-7} to 10^{-8} of the gas molecules by mass, and the organic components probably 10^{-3} of the entire colloid fraction. The problem is the sufficient and reliable concentration of material with which to work. I fully agree that chromatographic methods are most promising for this purpose, once the collection problem is solved.

Soffen — The sensitivities of gas chromatography now are 10^{-12} moles of some substances.

Yes, there appears to be good promise.

Solomon — Dr. Goetz, is it possible to collect these extremely minute aerosol particles through an appropriate device that will completely exclude larger particles. Let's say, with reference to the first point that you made, if one were sampling in an atmosphere that has spores or pollen grains in it, is it possible to exclude these larger particles by suitable methods and not change the properties of the "aerocolloids" that you were describing?

Goetz — You mean to single out microbiological matter from...

Solomon — From larger particles such as pollen grains or spores, something of the 10 to 30 μ range.

Goetz — Certainly.

Solomon — What device would you use?

Goetz — I would say again the best method to be centrifugal separation by the principle of the aerosol spectrometer because it effects a rapid fall-out in the low pressure range and a size-classified deposit that avoids particle interaction within the deposit.

Solomon — If one were to draw these through a molecular membrane filter?

Goetz — In our experience one cannot separate size classes of airborne particles with a cascade of membrane filters, neither can one avoid the danger of particle interaction.

On the other hand, the isolation of microbiological matter in a centrifugal field definitely may be worth thinking about because the act of particle separation and concentration by this principle causes probably the least chemical and physical harm.

Dahl — Dr. Goetz, in the case of the alfalfa example I recall that I can always smell an alfalfa field; the odor is due to coumarin. Do you have coumarin particles as a possibility here?

Goetz — I don't know. We noticed a strong smell from the deposit. As pollen or similar particles were excluded from the size range, it may be vaporized coumarin.

MacLeod — Dr. Goetz, did I understand correctly that the molar concentrations involved here are 10^{-13} and 10^{-14}? Does this indicate that you are talking about an organic coating of some particle rather than a droplet, so to speak?

Goetz — Yes, exactly: all evidence points to this at least as a realistic working hypothesis; namely that there exists an organic envelope on the

particulates, probably a product of a photochemical oxidation that upon hydration will be somewhat permeable to water vapor so that the particle can grow. It will grow slowly and upon reduction of relative humidity, the particle will shrink similarly at a retarded rate. We have speculated that this process causes the persistence of fog such as we have, for example, along the Southern California coast. Once such fogs have risen, they will persist even as far down at 40% relative humidity as dense hazes. They disappear slowly, just like these samples, because the organic envelope acts as a barrier to water evaporation. This is the Archer-LaMer phenomenon, whereby one can retard evaporation from water surfaces by extremely thin layers of many water insoluble organic substances.

MacLeod – Would one get a similar type of aerosol enclosed by some organic film as from an alfalfa field in transpiration?

Goetz – That, I don't know. The tests I showed were done in bright sunlight and none in the dark; hence, we don't know yet whether the effect of vegetable aerosols like those of alfalfa would be absent at night. Systematic experiments in this respect should still be done.

Divining for Water in Stratosphere

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Abstract

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Considerable controversy exists today as to the quantity of water that is in the upper atmosphere. The main area of contention is whether or not the measuring instruments are determining the water vapor of the stratosphere or water vapor brought up by the instruments as a contaminant.

A variety of instruments have been used to determine the quantity of stratospheric water vapor. Among these are: spectroscopic devices, conductivity elements, electrolytic hygrometers, microwave refractometers, dewpoint-frostpoint hygrometers, gravimetric samplers, and the hair hygrometer. A brief description of these instruments is given and their potential discussed.

Different investigators have reported widely divergent amounts of water vapor for the same altitude regimes. In general, the values appear to have a similar pattern, that is, the mixing ratio decreases with altitude to about 16 km and then increases at least to highest point measured to date, 36 km.

Humidity and temperature are two of the most important factors affecting the survival of microorganisms in the upper atmosphere. My life science associates inform me that the lower the relative humidity the greater the probability of survival in the stratosphere, that is, all other things being equal. Since water vapor is one of the main factors determining heat conditions in the stratosphere, it is quite obvious that information about the vertical distribution of water vapor in this zone is necessary for an understanding of its thermal structure. A knowledge of the vertical distribution of water vapor would aid in understanding the thermal equilibrium, radiation balance, and circulation of our atmosphere and aid in determining probability of finding microorganismic life in the stratosphere.

Research activity to determine the water vapor content of the atmosphere has increased considerably during the past decade, due mainly to the practical problems encountered by the military and space agencies. These are concerned with high-altitude flight of aircraft, rockets, and artificial earth satellites, infrared detection, radar tracking, and determination of the atmospheric composition of other planets.

Devices for Determining Water Content

A whole spectrum of devices have been and are being developed to determine the water content of the stratosphere. Among these "divining rods" can be found: hair hygrometers, conductivity elements, electrolytic hygrometers, dewpoint-frostpoint hygrometers, gravimetric samplers, spectroscopic and spectrometric devices, and microwave refractometers.

1. HAIR HYGROMETER. One of the earliest devices used, and in some areas still being used, is the hair hygrometer. This instrument operates on the principle of change of hair length with moisture content. An increase in humidity causes a related increase in hair length. These hygrometers are devices that respond slowly and they are unreliable. They cannot be used at temperatures below $0^{o}C$; they have only a $\pm 10\%$ calibration accuracy. They were used operationally, however, on radiosonde balloons in the United States until early 1943 and might still be used in some parts of Europe.

Since some of you might not be entirely familiar with radiosonde balloons, perhaps a brief description of this instrument would be helpful at this point. The radiosonde balloon is a small, helium-filled balloon to which are attached instruments for measuring pressure, temperature, and humidity and telemetering equipment for sending this information back to ground weather stations. These balloons, which the U.S. Weather Bureau releases at least twice a day from more than a hundred stations, rise to a height of approximately 30 km.

2. CONDUCTIVITY ELEMENTS. In February 1943, the U.S. Weather Bureau replaced the hair hygrometer in the radiosonde with a flat-strip, lithium chloride hygrometer (14). This instrument is essentially two tin electrodes separated by a film of lithium chloride. Lithium chloride is a dry powder

and a nonconductor below about 12% relative humidity. At higher humidities it takes up moisture from air to become a conducting film. The resistance of this conducting film varies with humidity and temperature. Over the years there have been some changes in design and preparation. In 1948, however, the design was essentially frozen; since then there have been only minor changes in the preparation of the hygrometer.

The flat-strip, lithium chloride hygrometer in its present design and preparation has a ± 3% calibration accuracy. It can be used to -40 C, only with difficulty. The element has a slow time response, particularly at the lower temperatures. For example, at -40 C it has a lag time of 120 to 480 seconds. The current altitude limitation for this hygrometer is 7 to 10 km, in contrast to the temperature and pressure elements which keep functioning to the scheduled balloon altitude (about 30 km).

Because of the slow speed of response at low temperatures of the lithium chloride hygrometer, studies were initiated in the mid-1940's to develop a faster responding hygrometer. These investigations involved replacement of the lithium chloride film with a carbon film (14). Because a great number of problems arose, the carbon element did not emerge from its long laboratory development until 1957. The first flights using the carbon hygrometer did not compare well with the lithium chloride device. Further flights, however, did show that the carbon element had a superior speed of response at temperatures below -15 C. In 1963, the U. S. Weather Bureau made the carbon hygrometer operational along with the lithium chloride element, in spite of the fact that the temperature and altitude limitations of the lithium chloride hygrometer also hold for the carbon type.

3. ELECTROLYTIC HYGROMETERS. Due to the inherent difficulties experienced with the hair, lithium chloride, and carbon hygrometers as operational sensors, the search has continued for a new operational sensor. These investigations have been concentrated on electrolytic, electrical resistance humidity elements. One that appeared to hold great promise was a polyelectrolytic film consisting of an ion-exchange resin (18). This polyelectrolyte consisted of a high polymeric cross-linked structure with polar groups of negative charge. Associated with these polar groups were ions of opposite charge which were held by electrostatic forces to fixed polar groups. In the presence of water vapor, water was absorbed and the electrostatically-held ions became mobile. These mobile ions were capable of electrolytic conduction when a voltage was applied across the resin.

This particular sensor did not meet with expectations. After a long laboratory study it had to be abandoned for a number of reasons: 1) it had a stability problem, i.e., its electrical resistance drifted with time, 2) its response time was only as good as the carbon hygrometer, and 3) it too was limited to temperatures above -40 C.

Experimentors are now determining the value of another sensor that utilizes an aluminum oxide film. It is too early yet to tell if the effort has been worthwhile.

4. DEWPOINT-FROSTPOINT HYGROMETER. The first systematic measurements of the water vapor content of the atmosphere above 10 km were made with a dewpoint-frostpoint hygrometer developed by Dobson (4). The instrument was flown in aircraft to altitudes up to 15 km and was manually operated. The measurements are based on the principle that the temperature of the bottom of the chamber through which the investigated air passes, rises or drops until equilibrium is established between the water vapor and the condensate.

This instrument's main measuring element is a small aluminum chamber, comprising a dish with a polished, anodized, and blackened internal surface. The bottom of the dish can be cooled from the outside with liquid nitrogen or heated electrically. When the temperature of the bottom drops to the saturation temperature, ice crystals grow upon it. The bottom of the chamber is illuminated so that as little as a microgram of ice on its surface can be observed with a magnifier. The temperature at which the amount of ice remains constant is taken to be the frost point.

Within the past decade, systematic measurements of stratospheric humidity have been made with balloon-borne automatic dewpoint-frostpoint hygrometers (1,3,6,7,13). These devices permit the determination of the vertical distribution of water vapor to altitudes of 30 to 35 km. Although there are several versions of the automatic dewpoint-frostpoint hygrometer used by different investigators, they are essentially based upon Dobson's instrument.

The improvements, in general, consist of incorporating in the instrument an optical-electronic-thermal servomechanism to maintain the temperature of the mirror (on which the moisture is precipitated) at the saturation level. This servomechanism controls the temperature of the mirror, within the limits of + 50 C to -80 C, with an accuracy of ± 0.2 C. A polished silver cylinder coated with rhodium usually serves as the mirror, while a coil wound on the mirror serves as the heating element. The end of the cylinder is lowered into a bulb of liquid nitrogen, whose pressure is regulated by the servomechanism.

The servomechanism, itself, is controlled by the amount of light striking two photocells, one after reflection from the mirror and the other directly. The amount of light reflected by the mirror depends upon the thickness of the frost film. A bridge circuit at a fixed thickness of film balances the two photocells. Thus, an increase in the film thickness turns on the heater, while a decrease opens a valve to the bulb with liquid nitrogen. The water vapor contained in the chamber is, therefore, constantly in equilibrium with the solid or with the liquid phase.

Recent models of these instruments have incorporated ventilating units and Peltier coolers. The ventilating units can completely remove the condensate from the measuring chamber at will. This removes some of the possible error that was inherent in the earlier devices. Peltier coolers are semiconductor devices that can both heat and cool, thus eliminating the necessity for heating coils and liquid nitrogen baths. The Peltier device has simplified design and construction of such instruments

as these. Within the past few years, Ballinger (1) has replaced the optical system with an alpha-radiation device to control film thickness. Since this device will be discussed by the next speaker, I shall pursue it no farther.

5. GRAVIMETRIC SAMPLERS. Another class of water-vapor measuring instruments has been developed during the past ten years, the gravimetric type. Goldsmith and his associates (2,5) developed a liquid nitrogen trap that samples air at fixed altitudes up to 30 km. In this instrument, air is passed through a tube cooled with liquid nitrogen, and the water vapor and carbon dioxide in the air are frozen out. The sampling unit is returned to the laboratory for quantitative measurement of water vapor and carbon dioxide. Assuming a constant carbon dioxide content of air at 0.031%, one can calculate the amount of air sampled, and in this way determine the mixing ratio of the water vapor.

More recently, an adsorption-type of gravimetric sampler was developed by General Mills' personnel, now members of the Applied Science Division of Litton Systems, Inc. (17,20,21). In connection with the development of this technique I have made experimental balloon flights utilizing this type of collector. Our sampling devices utilize the principle of adsorption of water vapor and carbon dioxide on synthetic zeolites. Two different samplers for use at two different altitude regimes have been developed using the adsorption technique. One sampling unit which can be flown to an altitude of approximately 30 km, has a blower that draws ambient air through two beds of zeolite (see schematic diagram, Fig. 1). The other sampling unit, which is useful above 30 km, has a cryogenic adsorbent pump that draws air through a bed of zeolite. This pump can also be used as a whole-air sampler (see Fig. 2, schematic diagram). As in the Goldsmith trap, the sampling units are returned to the laboratory for quantitative analysis of water and carbon dioxide.

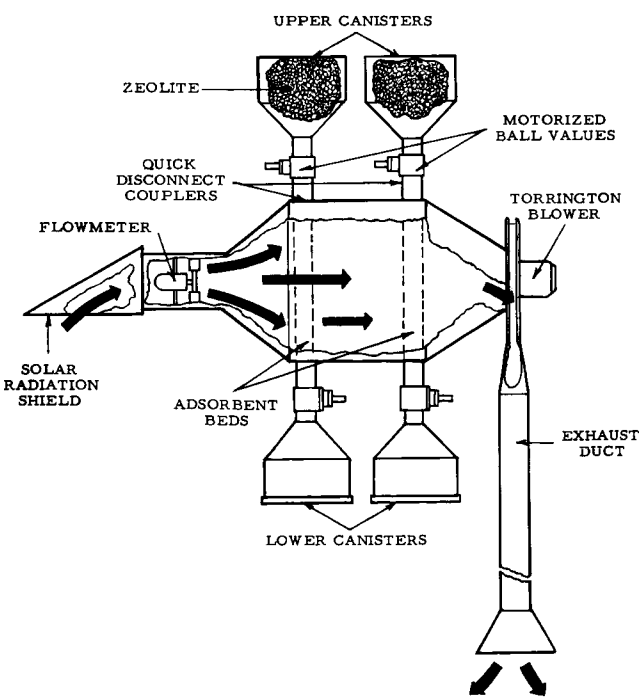

Fig. 1. Gravimetric adsorption sampler for altitudes to 30 km.

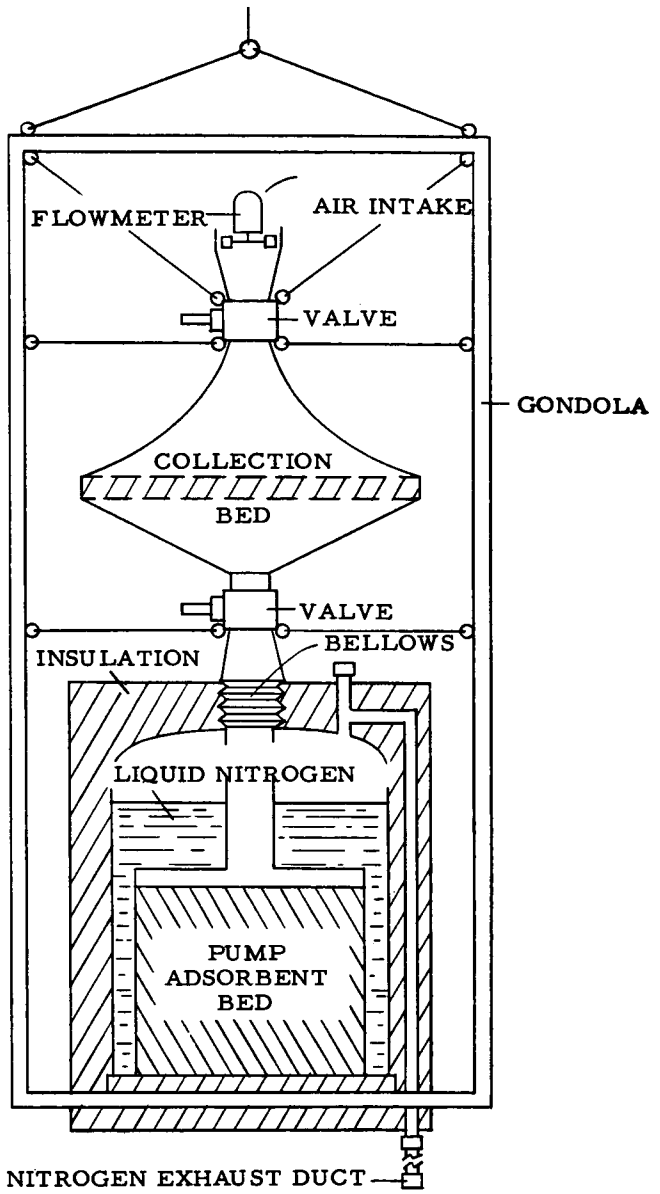

Fig. 2. Cryogenic adsorption pump sampler for altitudes above 30 km.

In our flights (see Figs. 1 and 2) we included a flowmeter to determine the air volume sampled. In this manner we determined the carbon dioxide concentration at altitude or checked the flowmeter volume of air by assuming a constant concentration of carbon dioxide, as Goldsmith did. The carbon dioxide concentration appeared to be constant in the stratosphere at an average value of 0.031% by volume.

The major advantage that the zeolite adsorption units have over the liquid nitrogen trap is that a much greater volume of air, approximately 50 times greater, can be sampled in a shorter period of time. In general, the gravimetric systems have the disadvantage of sampling at one altitude only, as opposed to frostpoint-dewpoint hygrometers that give a vertical distribution. Gravimetric samplers have the advantage, however, of obtaining samples of water and carbon dioxide that can be analyzed for isotopic content, such as deuterium, tritium, and carbon 14.

6. SPECTROSCOPIC AND SPECTROMETRIC DEVICES. Water vapor has a series of intense absorption bands in the infrared and a series of rotational bands from 20 μ to the microwave region. Therefore, measuring the sun's spectrum at different heights in the atmosphere and at various resolutions makes possible a determination of the water vapor content in the layer located above the level at which the measurement is carried out. Measurements have been made using the sun's infrared spectrum from airplanes and balloons (10, 12,15,16,22). In these studies, both prism and grating instruments have been used. The image of the sun is guided onto spectrometer slits by means of a heliostat. The heliostat is a device that can follow the sun and reflect its light into the spectrometer. The interpretation of the spectra is difficult mainly because of the insufficient resolution of the instrument and errors determined by function of the apparatus, such as improper heliostating, changes in air pressure, and partial pressure of water vapor, etc.

Infrared hygrometers, which have their own radiation source and do not depend upon the sun, are currently being developed to measure water vapor at altitude. Since they are not completely ready for use at present, I shall discuss them no farther.

Another type of spectrometer humidity-measuring instrument is the Lyman-alpha humidiometer (9). This device is based upon the fact that water vapor has a large coefficient of absorption of Lyman-alpha radiation (1,215.6 A) produced by hydrogen. Instruments utilizing this principle have been flown on aircraft and even on rockets.

7. MICROWAVE REFRACTOMETERS. The last type of instrument I should like to discuss briefly, in the category of instruments that have been flown experimentally on balloons, is the microwave refractometer. The microwave index of refraction of air depends upon atmospheric pressure, temperature, and partial pressure of water vapor. Thus, if temperature and atmospheric pressure can be obtained independently, then the water vapor pressure can be determined from measurements of the index of refraction. The presently available index of refraction sensors utilize the same principle, i.e., they are capacitors that have an operating frequency in the microwave region. At present, these instruments have poor accuracy above 10 km. Further studies are being made to improve them.

Discussion

Now that I have briefly described some of the measuring techniques, what values have been obtained for the vertical distribution of water vapor in the atmosphere? A comparison of flight data, in terms of mixing ratio, obtained by various investigators is given in Figure 3. This figure is a modified version of data compiled by Gutnick (11). For those unfamiliar with the term "mixing ratio," it is defined as follows:

$$\text{Mixing ratio} = \frac{\text{Weight of water vapor (g)}}{\text{Weight of air sampled (kg)}}$$

The mixing ratio and the frostpoint and dewpoint temperatures are used by most investigators today to indicate the amount of water present.

There is a scattering of values for the same altitude (Fig. 3). The values appear to have a similar pattern, however, i.e., the mixing ratio decreases with altitude to about 16 km and then increases at least to the highest point measured to date, 36 km. Incidentally, this value was obtained with the cryogenic adsorption pump device.

A great controversy has arisen as to whether the values above 16 km are true or whether they are indicating water vapor brought up by the measuring instrument or balloon as contamination. British investigators emphatically assert that the values obtained above 16 km are contamination and that the water vapor content of the atmosphere above 16 km is either constant or decreasing. They have calculated that a contaminated instrument containing a film of water a few molecules thick can act as an infinite source of contamination. Some American researchers feel that the atmosphere has a variable water vapor content. In fact at the recent 1963 International Symposium on Humidity and Moisture at Washington, D.C., there was a clear division of opinion with the divergent groups being called either the "wets" or the "drys."

Although the differences in values appear to be of insignificant amounts, these differences have both practical and theoretical implications. In the practical area one finds that even a minute amount of water vapor can make the difference between success or failure. The infrared detectors might not detect the radiation emitted by intercontinental ballistic missiles. Certain theories about the circulation in the stratosphere are based upon some of the observed values; if these values are incorrect then the theory is invalid.

Direct evidence for the existence of high, localized concentrations of water vapor in the stratosphere are the noctilucent clouds that have been observed at 75 km. On the cover of the June 1963 issue of Scientific American is a photograph of a noctilucent cloud over Sweden. These clouds have been found over that part of the country quite often. Rocket samplings have shown that such clouds contain ice crystals (19).

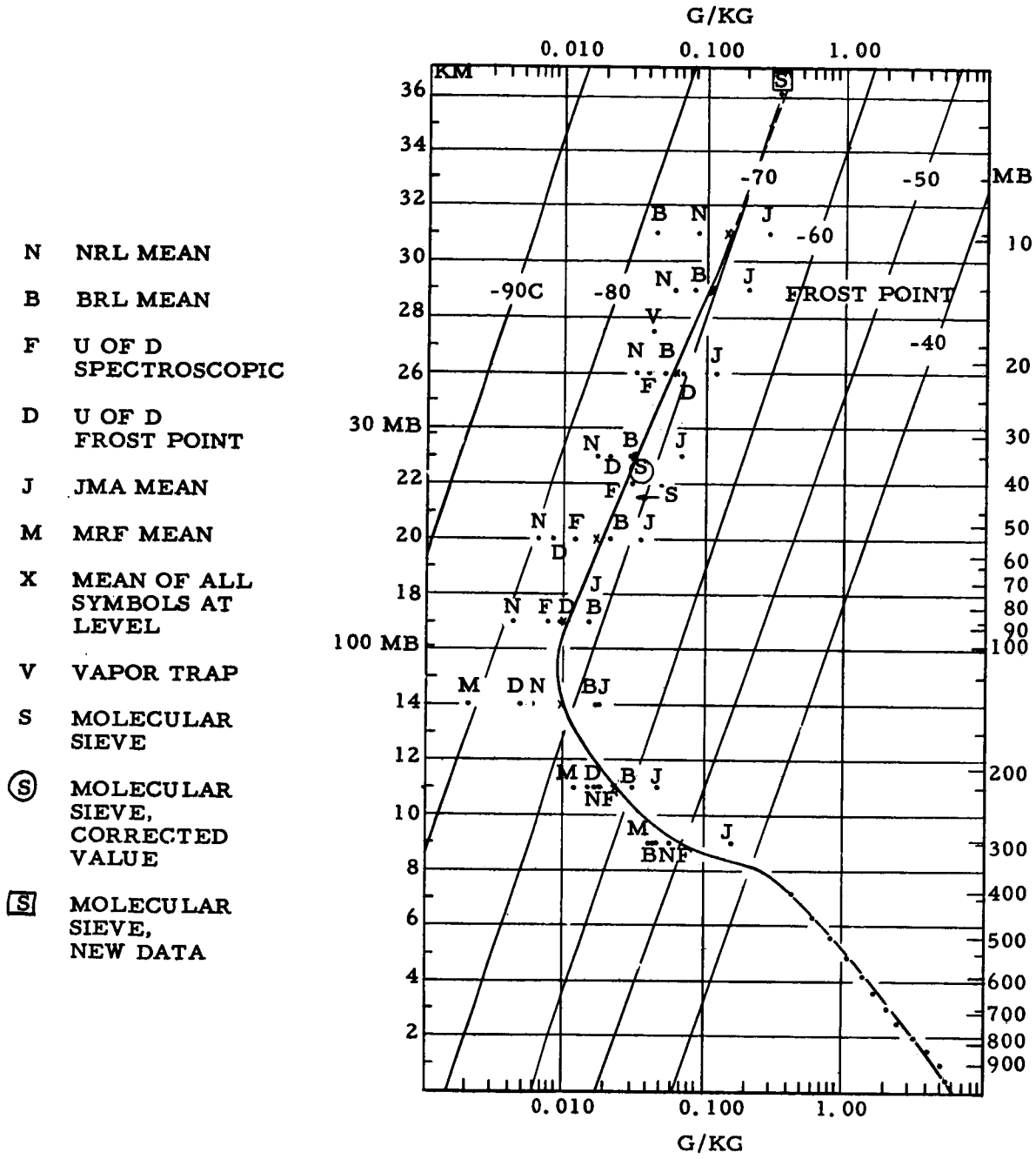

Fig. 3. Comparison of flight data with mixing ratios obtained by various investigators (modified from Gutnick, 11).

Further evidence for the possible existence of high, local water vapor content in the stratosphere is the presence of nacreous clouds at 22.5 to 24 km. These clouds appear in high-temperature latitudes, generally over mountain ridges. Such a cloud, photographed over Arizona, appears on the cover of the 19 April 1963 issue of Science. This cloud has a queer ring structure. Although the possibility exists, it has not been proven that nacreous clouds are composed of water. The presence of such a cloud does show, however, that large concentrations of material can penetrate into the stratosphere.

How both types of cloud reach their respective altitudes or why they exist there are at present unknown.

From thermodynamic considerations, it is practically impossible for water vapor from the earth's surface to rise above approximately 16 km. Whether water found in the stratosphere is due to chemical or photochemical composition is presently unknown. Transport phenomena that have been suggested are advection and convection. Present evidence, however, does not wholly support these processes.

About 3 years ago, DeTurville (8) suggested that water vapor is formed at altitudes above 100 km, from the proton flux that had been emitted from the sun and captured by the earth. His rough calculations show that complete oxidation of the hydrogen captured throughout the existence of the earth might account for the earth's total ocean mass. If this theory is correct, then it can explain the shape of the vertical distribution curve. Continuous formation of water vapor at altitudes above 100 km must lead to a decreasing downward gradient of mixing ratio until it is met by the decreasing upward gradient arising from evaporation of water from the surface of the earth. DeTurville's postulation, however, leaves many questions unanswered, but it is an interesting possibility.

Conclusions

It is obvious from what I have said that a great deal of investigation must be performed. Studies must be carried out to prove or disprove the possibility of erroneous results due to contaminated equipment. Furthermore, flights should be performed with a variety of measuring devices at the same time and place to correlate the operating accuracies of the various types of sensors. Only through extensive laboratory and field studies can the controversy between a "wet" and "dry" stratosphere be settled.

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Discussion

Junge — If I may just make a remark about this idea of water vapor coming from outside. It cannot be upheld against some pretty good evidence that oxygen on our atmosphere during the time of evolution has apparently increased rather than decreased. In case water is formed by the influx of protons one would, of course, expect decreased oxygen. This can be rather well ruled out on the basis of geochemical evidence.

Also, there is no necessity to assume hydrogen influx because the increase of the mixing ratio with altitude can be explained on the basis of certain mechanisms of vertical mixing. For instance, as Brewer (A) stated in 1949 (and I think up to now,

fairly correctly) that the dewpoint around -83 C, always found around 15 km, is due to the cold-trap action of the tropical troposphere. One would assume that the effect of this cold trap would somehow become less efficient if one goes to higher latitudes. So it could well be that by some vertical motions in the polar stratosphere (particularly the polar night stratosphere) some parcels of air with higher mixing ratio can penetrate through this dry layer around 15 km from below, and penetrate into the higher atmosphere.

New data that were recently obtained by H.J. Mastenbrook in the Naval Research Laboratories with his new radiosonde, seem to indicate that the older data are wrong and that the mixing ratio stays constant with altitude. He thinks that the older measurements reflected contamination. Mastenbrook has excluded contamination by measuring on the way down with the instrument located below the balloon. He found constant mixing ratios in a number of flights and I must admit his results look good, but he has still to explain how the older data were so consistent, particularly the data obtained by spectroscopic measurements. It is hard to understand how the contamination around the balloon will affect the whole light path from the balloon instrument to the sun. The whole subject is absolutely open for discussion at the moment. All we can do is wait for better data.

Steinberg — I agree with you wholeheartedly, Dr. Junge; in fact, it is the premise of my talk. More data are needed to iron out these difficulties we are having. Perhaps the difficulty isn't conflicting data; perhaps it is a variable atmosphere. I do not know.

I have spoken with Mastenbrook at a couple meetings; I have this feeling that he is changing his mind about his earlier data. Also he apparently is having, I understand, some difficulty with his instrument. He has been obtaining dewpoints at much lower temperatures than most people believe his device can measure. Even Brewer questions his -90 C dewpoint.

Contamination is a big problem. How would the contamination in spectrometry explain the complete path? It is difficult to understand how contamination will explain some of our data; we've taken a large sample, thinking that a large sample would seem to overshadow the contamination. I do not know. We took precautions against all possible contamination by including drying devices on our instrument packages and starting the blowers ahead of time in order to pull out any contamination that may have arisen during the flight. Of course we can use a lot more experimentation. The possibility does exist that there is possible contamination in all cases.

More work has to be done. More comparative flights are needed at the same time between different instruments measuring the same air. Perhaps that way we can determine if we are getting pure samples.

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Stratospheric Frost-Point Measurements Using the Alpha Radiation Hygrometer

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Abstract

Associated with reliable frost-point measurements of the stratosphere are problems of proper instrument operation under low frost-point conditions and contamination from extraneous sources of water vapor. These problems are discussed and results of two night balloon flights from Sioux Falls, S.D., are presented. These flights had several models of alpha radiation hygrometer (Honeywell) on board. Results obtained with these units indicate little difference between ascent and descent frost points. Present programs in this field are discussed.

I. Introduction

The total precipitable water and the dew or frost point in the stratosphere are of great importance for many reasons. Estimates of world air-mass movements are of great interest to meteorologists for weather forecasting. Guidance and tracking of rockets require accurate knowledge of the atmospheric index of refraction, which varies with water-vapor content. Many of the people at this conference are interested in the stratospheric water content as it pertains to life support for microorganisms.

Two serious difficulties exist in determining the amount of water vapor in the stratosphere. 1) The low concentration of water vapor may reduce the rate of mass transfer to the mirror surface of automatic frost-point hygrometers to such an extent that the servo control system becomes ineffective. 2) Since moisture is carried into the stratosphere by the balloon and instrument package the accuracy of the measurement of ambient frost point depends upon how much of this excess moisture is desorbed into the sampling region.

In November 1963, Honeywell alpha radiation hygrometers were flown on two night-launched balloon flights. The alpha hygrometer determined the frost point by maintaining a constant amount of frost deposit on a cooled radioactive surface; the thickness was sensed by the attenuation of alpha particle energy. The steps taken to reduce contamination, the results of the flights, and some conclusions are presented in the following sections. In addition, the operation of the alpha hygrometer is briefly described.

II. Background Information

Investigation of water vapor was restricted for many years to tropospheric measurements of dew points and relative humidities. Ground-based infrared devices aimed at the sun measured the total precipitable water in the atmosphere. Since there are large variances in ground and tropospheric water vapor, however, these measurements gave only clues as to the water content of the stratosphere.

Direct investigation of the water-vapor content was extended to the upper atmosphere more than 15 years ago. Early work by Dr. A.W. Brewer and co-workers (3) in England resulted in the development of a manually operated optical hygrometer suitable for stratospheric measurements from aircraft. Data obtained with this type of unit was necessarily limited at that time to altitudes of less than 55,000 ft.

The collection of water samples by balloon-borne systems, with the resultant single-point determination of water-vapor mixing ratio, extended the information range to nearly 100,000 ft. Early systems collected a large sample of air in a polyethylene balloon. Upon recovery of the balloon the collected air was compressed into cylinders for later analysis. Freeze-out traps that utilize a coil immersed in liquid nitrogen to remove the water vapor from the air sample were developed by the British Meteorological Group (2). The air sample was drawn through the tube, and the water was frozen in the tube. The tube was sealed at the sampling altitude, reducing the contamination problem during handling on the ground. The small tubing and blower on this unit resulted in low volume flows with correspondingly long sampling periods. The adsorbent system (4) of water collection could sample higher volume rates, thereby reducing collection time. A high-volume blower drew the air sample through an adsorbent bed that collected the

water that was in the air sample. The adsorbent was then sealed at altitude and returned to ground level, again reducing the handling problem. With each of the above systems one can obtain the mixing ratio at only one point.

The development of the automatic hygrometer for obtaining frost or dew points enabled data to be collected continuously from ground level to the maximum altitude of the carrying vehicle (balloon or aircraft). Automatic hygrometers that optically detect changes in the reflectivity of the frost layer have been designed by many investigators. These instruments, generally flown on balloons, sample the atmosphere continuously, either telemetering the frost point data to a ground station or recording it on board the package for later analysis.

Automatic hygrometer data obtained by various observers at many locations have yielded radically different values of stratospheric frost point. This has resulted in conflicting points of view and a current controversy as to the amount and distribution of stratospheric water-vapor content. Although several basic meteorological reasons for this divergence of data have been postulated, the divergence is most probably attributable to operational failure of frost-point hygrometers as a result of low mass transfer rates and errors caused by the excess water desorbed from the instrument package and associated equipment.

To date, little is known about the frost-formation, mass-transfer rates, etc., that occur on and around the frost formation surface of a hygrometer. Steady-state conditions at atmospheric pressures have been examined, but the effects of sudden changes in frost point or changes in the ambient pressure have not been experimentally determined. Since mass transfer rates are low, under low frost-point conditions, the frost-point indication from an automatic instrument can be either very sluggish or can undergo large oscillations, depending upon the manner of servo control. Obviously this can lead to false measurements.

Instrument contamination is also considered an important problem in determining atmospheric water-vapor content; the effect of contamination must be carefully considered. Measurements made in the balloon wake can be erroneous due to contamination from water that has been "stripped" from the balloon and from the package surfaces as the package is drawn through this contaminated air on ascent. Solar heating of the equipment can cause desorption of water from all surfaces of the equipment. The flight system must be examined for possible sources of contamination; these sources must be either eliminated or accounted for in the data analysis.

III. Operation of Alpha Radiation Hygrometer (1)

Previous automatic frost-point hygrometers used for stratospheric measurement employed changes in the light reflectivity of a cooled-mirror surface to indicate changes in the condensate on the surface. The alpha radiation hygrometer uses the fundamental approach for determining frost thickness by energy attenuation of alpha particles that pass through the layer.

One advantage of this approach is that the nature of the dew or frost layer (which can change under different conditions of formation at different altitudes) has little effect upon the measurement.

In our experiments two alpha radiation sources (Po^{210}) emitted 5.3 Mev alpha particles (see block diagram, Fig. 1). One of the alpha sources was fastened to the cold junction of a thermoelectric cooler and coated with a thin gold overlay. The thermoelectric cooler was mounted on a heat rejection surface. Particles from the first alpha source passed through the frost layer and were detected by a solid-state, alpha-particle detector. Particles from the second source passed through a control-layer absorber and were detected by a second detector. All alpha particles passed through the absorbing media with a resultant attenuation in energy. While changes in thickness of the frost layer did not, in general, result in changes in the pulse rate, N_O, received by the preamplifier, they did result in changes in the pulse energy distribution. An electrical threshold level was set at the flip-flop so that only the higher energy pulses could pass. Thus, a change in frost-layer thickness caused a resultant change in the frost-layer pulse rate, N_F, beyond this electrical gate. This same electrical threshold was applied to the reference channel, and the pulse rate, N_R, passing this gate determined the frost-layer-thickness control point about which the instrument cycled. The reference channel had no frost layer and thus provided automatic correction for changes in density of the air gap between source and detector — which is important for stratospheric flights.

Two random trains of input pulses to the flip-flop and the resultant output waveform are shown in Figure 2. The reduction in pulse height, as a result of absorption in the frost and control-layer absorber, is shown together with a typical electrical gate setting. When a sufficiently large pulse occurred in one channel, the flip-flop was switched to one state and remained in that state until a pulse from the other channel drove it to the other state. Since the input pulses were entirely random, the flip-flop switching was also random.

The long-term output voltage from the flip-flop as it swung between states was related to the pulse rates, N_F and N_R, and can be written

$$\bar{V} = \frac{N_F - N_R}{N_F + N_R} V$$

where N_F = Frost channel long-term-average counting rate

N_R = Reference channel long-term-average counting rate

V = Flip-flop state voltage.

This voltage, when integrated and amplified, became the mean error signal that drove the thermoelectric cooler to the balance condition.

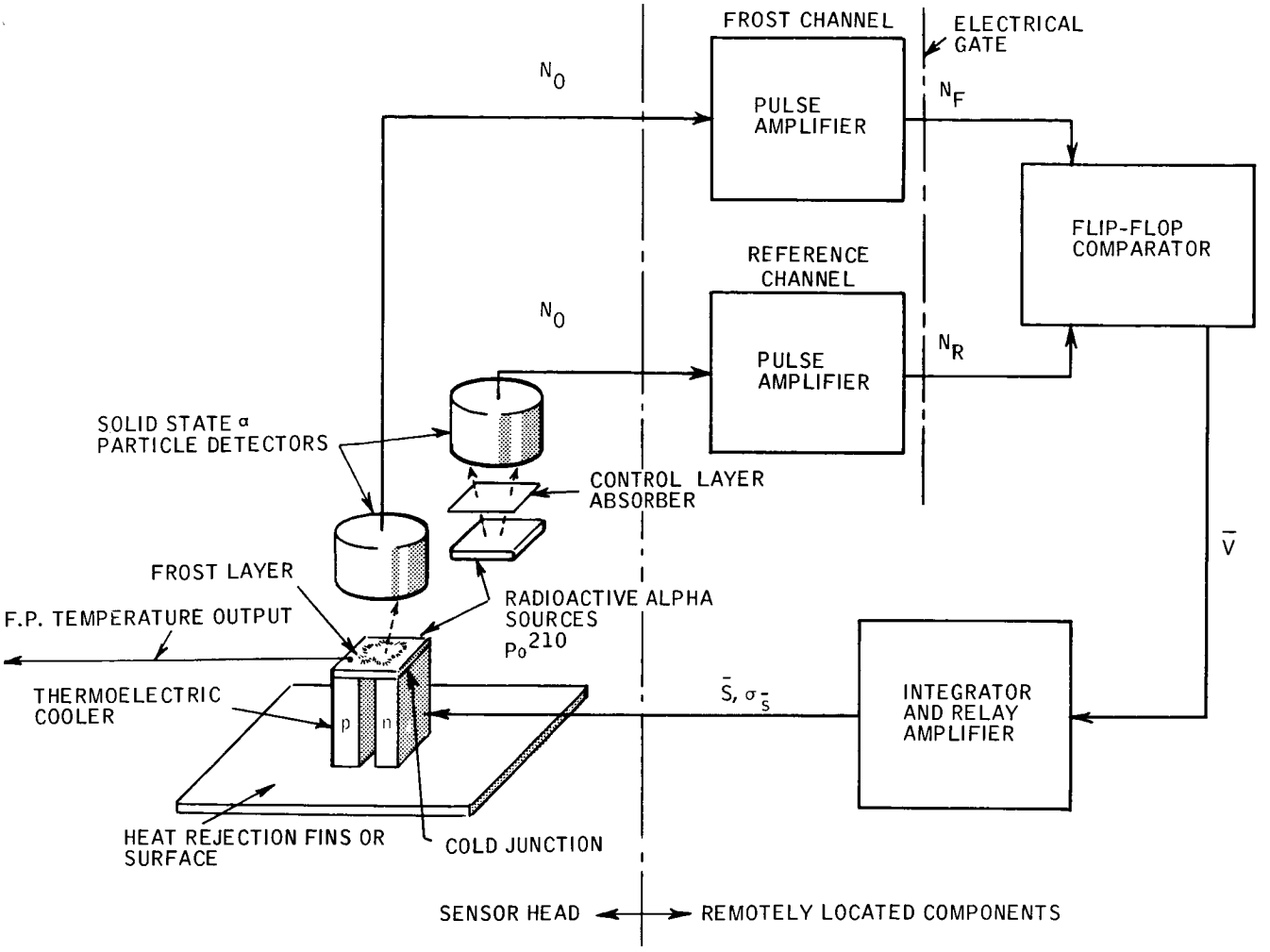

Fig. 1. Block diagram of alpha radiation hygrometer. (Originally shown at 1963 International Symposium on Humidity and Moisture, Washington, D. C., and published in "Humidity and Moisture," Vol. I. Reinhold Publishing Co., New York, 1964. Reprinted by permission of editor-in-chief.)

In a linear approximation, this error signal could be considered proportional to the difference between the frost thickness, σ_F, and an equivalent thickness, σ_R, of the absorber in the reference channel. This difference then determined the value of the current to the thermoelectric cooler, which in turn could be considered proportional to the temperature T of the cooled surface. If the error signal was integrated, then

$$\frac{dT}{dt} \propto (\sigma_F - \sigma_R),$$

and if not integrated, then

$$T \propto (\sigma_F - \sigma_R).$$

In either case, for $T \neq T_o$, where T_o is the frost point, then

$$d\sigma_F/dt \neq 0,$$

and the change in $(\sigma_F - \sigma_R)$ drove T to the balance condition: $T = T_o$. During this servo operation of the instrument, T was continuously monitored and was in general an oscillating function in time about T_o. The extreme sensitivity of the alpha absorption technique in sensing minute changes in frost-layer thickness led to fast response and cycling characteristics of the servo system. These characteristics had crucial significance in stratospheric applications where the rates of frost formation and sublimation that were obtained for a given difference T and T_o, were severely reduced from those that occurred near the earth's surface.

IV. Recent Flight Results

Two balloon flights were planned for November 1963 under co-sponsorship with Air Force Cambridge Research Laboratories. The purpose of these flights was to obtain stratospheric frost-point data. The flight package was carefully prepared to keep the amount of contamination to a minimum. A sealed

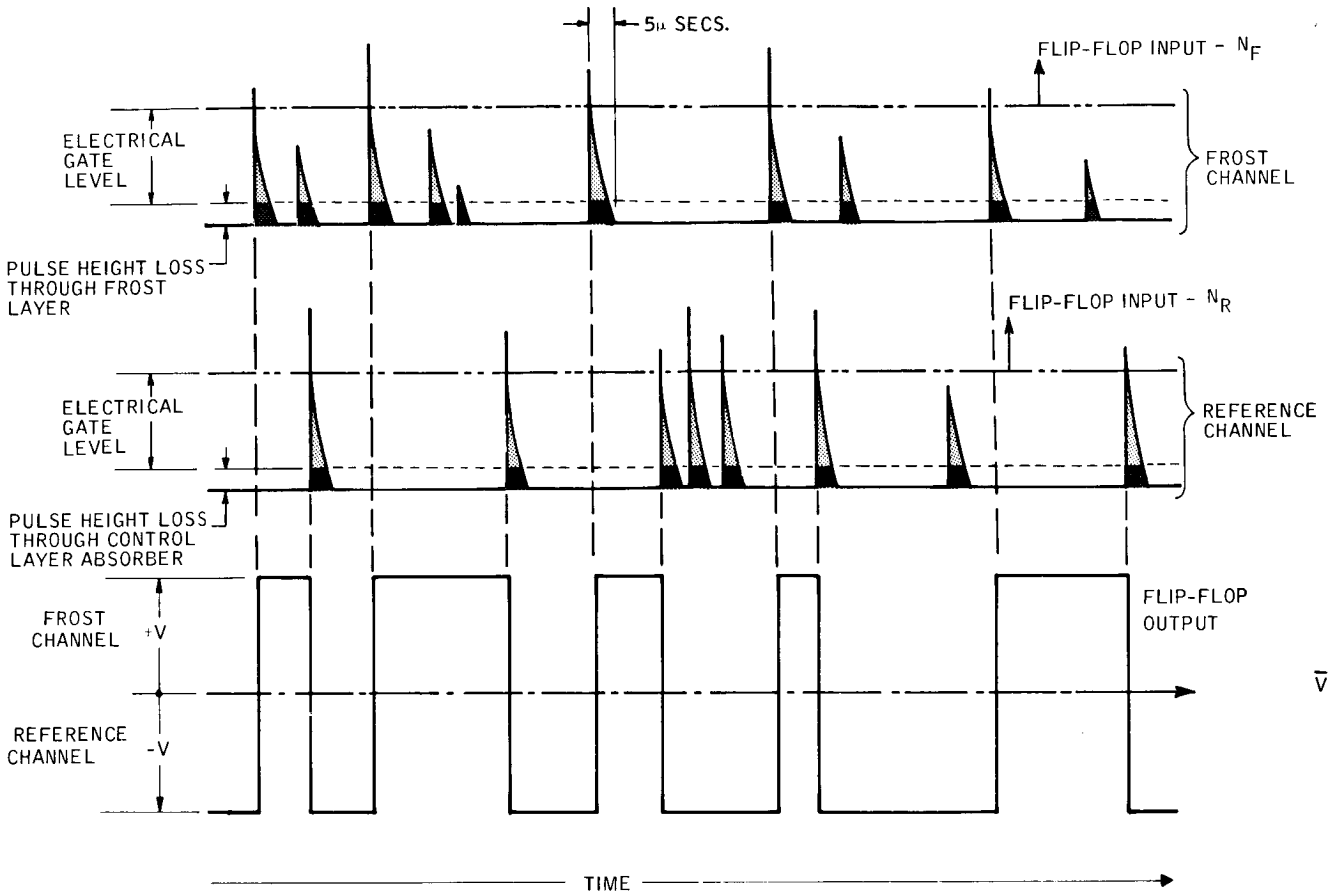

Fig. 2. Flip-flop input and output waveforms. (Originally shown at 1963 International Symposium on Humidity and Moisture, Washington, D. C., and published in "Humidity and Moisture," Vol. I. Reinhold Publishing Co., New York, 1964. Reprinted by permission of editor-in-chief.)

container held the batteries and equipment for telemetering the frost-point temperature, free-air temperature and pressure, and servo monitoring of one of the frost-point hygrometers. In order to reduce the surface area available for water adsorption, no crash pad material was used. The sealed container was placed at the center of a 3-ft skeleton cube. The frost point hygrometers were located such that their sensing heads were in the air stream outside the 3-ft cube. On-board recorders provided a backup of the telemetered data for four of the hygrometers.

There were some differences between the units flown on the two flights. Two of the units were the research models, type S; the remaining units were engineering models, type G. In both models the normal heat sink was a 1-ft^2 fin. This fin conducted heat from the Peltier cooler and radiated it to the atmosphere. One of the G-type units, however, had a heat-of-fusion sink that consisted of ethyl alcohol (freezing point, -114 C) in a sealed container and was designated type F. The container of alcohol was frozen in liquid nitrogen. It was planned that both ascent and descent data would be obtained on each flight to check the effect of balloon-wake contamination. The ascent and descent rates were alike to provide similar ventilation conditions for the hygrometers on both phases of the flight.

Both flights were made at night to eliminate the effects of solar heating of the equipment with the associated desorption of water. The balloons were launched from Sioux Falls, S. D. Due to strong easterly winds in November, a second ground-tracking station was established at the Honeywell Ordnance Plant in Hopkins, Minn., as a backup should the balloon travel out of range of the ground station used for launch. In this manner data would be obtained from the time of launch until the tropopause was reached on descent.

The first flight was launched at 1849 CST on 17 November 1963 (M-H flight No. 9). The payload of 676 lb included 120 lb of ballast. The ascent rate averaged 450 ft/minute to 30,200 ft, and 415 ft/minute from this point to the float altitude of 95,500 ft. The system was allowed to remain at the peak altitude for about 60 minutes before descending at 434 ft/minute to 50,000 ft. At this point, all telemetering data ceased due to battery failure.

Because of a malfunction in the information switch, the data from the heat-of-fusion instrument (F-1) was lost. The G-7 instrument had a malfunction that caused a severe drain on its battery; its data were lost shortly after launch. Data were obtained, however, from three units.

S-2, Which was the most sensitive hygrometer, transmitted data until the balloon reached float altitude (Fig. 3, *solid line*). A sharp, dry layer with a frost point of -44 C was noted at about 640 mb and a moist layer with a frost point of -30 C at 600 mb. This instrument showed good cyclic action from approximately 130 mb to the float altitude of 13 mb. The frost point remained nearly constant at -75 C. Unfortunately no descent data were obtained on this flight for a comparison to determine the effect of the balloon wake.

The second research model, S-1, worked during the first portion of the flight. These data (Fig. 3, *dotted line*), followed quite well that of hygrometer S-2, but did not show as distinctly the sharp dry-moist region near 600 mb. This was due to the fact that S-1 was not as sensitive as S-2. Data are shown only to an altitude of 250 mb since at this point a malfunction caused the instrument to remain in a full-heating mode and the data were lost. The third curve (Fig. 3, *dashed line*) represents data from the G-10 instrument. This unit was off at launch and was not turned on until about 590 mb. At this point the instrument achieved a frost deposit and followed unit S-2 to 250 mb; at this point the high drain on the batteries caused it to fail. The data for G-10 has been smoothed on Figure 3 to make it easier to discern the data from each instrument. The same cyclic action was noted on all instruments.

This flight was considered as only a partial success since there was no verification of the frost point data obtained by S-2. A servo monitor placed on this hygrometer, however, indicated that the unit was operating throughout the flight.

Since no damage occurred at impact, the package was readied for the second flight. Wiring changes were made and the batteries grouped to provide longer life for the G-series hygrometers.

At 2336 CST on 23 November 1963, the second flight was launched (M-H flight No. 10). The load was 691 lb with 110 lb of ballast. A more rapid ascent was established on this flight with an ascent rate of 735 ft/minute to the peak altitude of 97,960 ft. It was hoped that the higher ascent rate would allow completion of the flight before the batteries discharged. Upon reaching the float altitude, the balloon system immediately began descending at 270 ft/minute until 74,000 ft. Thereafter, the descent rate was 367 ft/minute until signals were lost at 65,000 ft. A short circuit in the dc-to-dc converter terminated the data transmission.

In Figure 4 are the ascent data of the second flight. S-2 Again showed the dry-moist region at 500 mb. The change this time was slightly larger, 17 C, than on the first flight where the change was 14 C. The general pattern, however, was the same as on the first flight; above about 80 mb the frost point was again nearly constant at -75 C.

Hygrometer S-1 was off at launch and turned on by radio command at an altitude of 780 mb. It immediately went on full cooling to form its frost layer; it overshot the frost point and then began tracking at about 700 mb. From this point on, S-1 again indicated the same frost-point values as S-2, with a lack of sensitivity similar to that indicated on the previous figure. The data was again lost at approximately 210 mb by the same effect that occurred on the first flight. Both G-series instruments, G-7 and G-10, lost their frost layers immediately after launch. As a result, they both were on full cooling until they could recover and form their frost layers. This occurred for G-7 at approximately 250 mb and for G-10 at 210 mb. From this point on, both instruments attempted to measure the frost point.

At launch, hygrometer F-1 had a heat-sink temperature of -196 C. The Peltier cooler on this unit could not produce enough heat to raise the temperature of the frost-formation surface to the true frost point. At 300 mb the frost point was low enough that the hygrometer could operate. The unit then followed the same general curve as S-2 from this point up to float.

Disagreement in the readings of the instruments at high altitudes was readily accounted for by the following uncertainties: 1) errors in thermistor calibration could contribute discrepancies of up to ± 2 C; 2) sluggishness of the servo systems due to poor mass transfer at low frost points could produce an apparent balance for many minutes while the frost temperature was actually up to 3 C higher or lower than the frost point, and when the frost point changed in time this offset could occur continuously; 3) inadequate ventilation of the sampling region could cause the measured frost point to lag the ambient value, that decreased in time on an ascent flight.

The ventilation of units G-7 and G-10 was particularly poor. G-7 Formed its frost layer and came into equilibrium with the air around the sensor at a relatively moist frost point. This was the most probable reason for the more pronounced divergence of hygrometer G-7.

In Figure 5 are shown the complete ascent data and the descent data of S-2 to 58 mb as well as the descent data of G-7. While this flight did not float for any period of time, it should be pointed out that the period from 20 mb on ascent to 20 mb on descent was actually a long time (75 min) due to the slowing down and speeding up of the balloon as it reached altitude and began to descend. There was only a slight change over the first 20 mb in the indicated frost point, however. In addition, the descent reading at 58 mb was only about four degrees Centigrade colder than that recorded on ascent. Some contamination was expected on this flight; apparently the contamination was no greater than that which would produce a 4 C difference. G-7 Indicated a descent frost point that was approximately seven degrees Centigrade warmer than the S-2 descent values. This could again be attributed to poor ventilation; the excess moisture accumulated on ascent was not completely flushed away from the sensor.

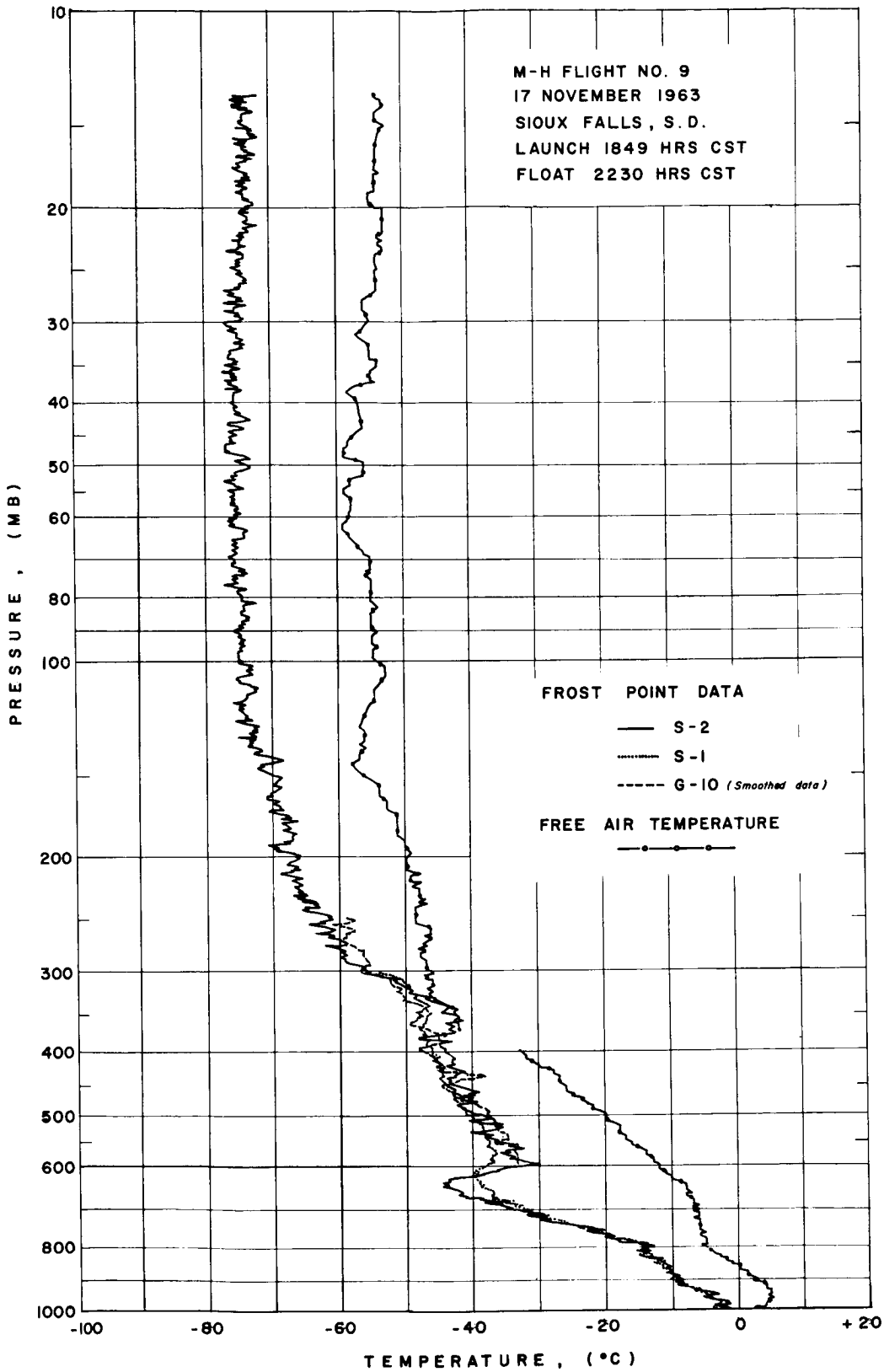

Fig. 3. Ascent frost-point profile, 17 November 1963 flight.

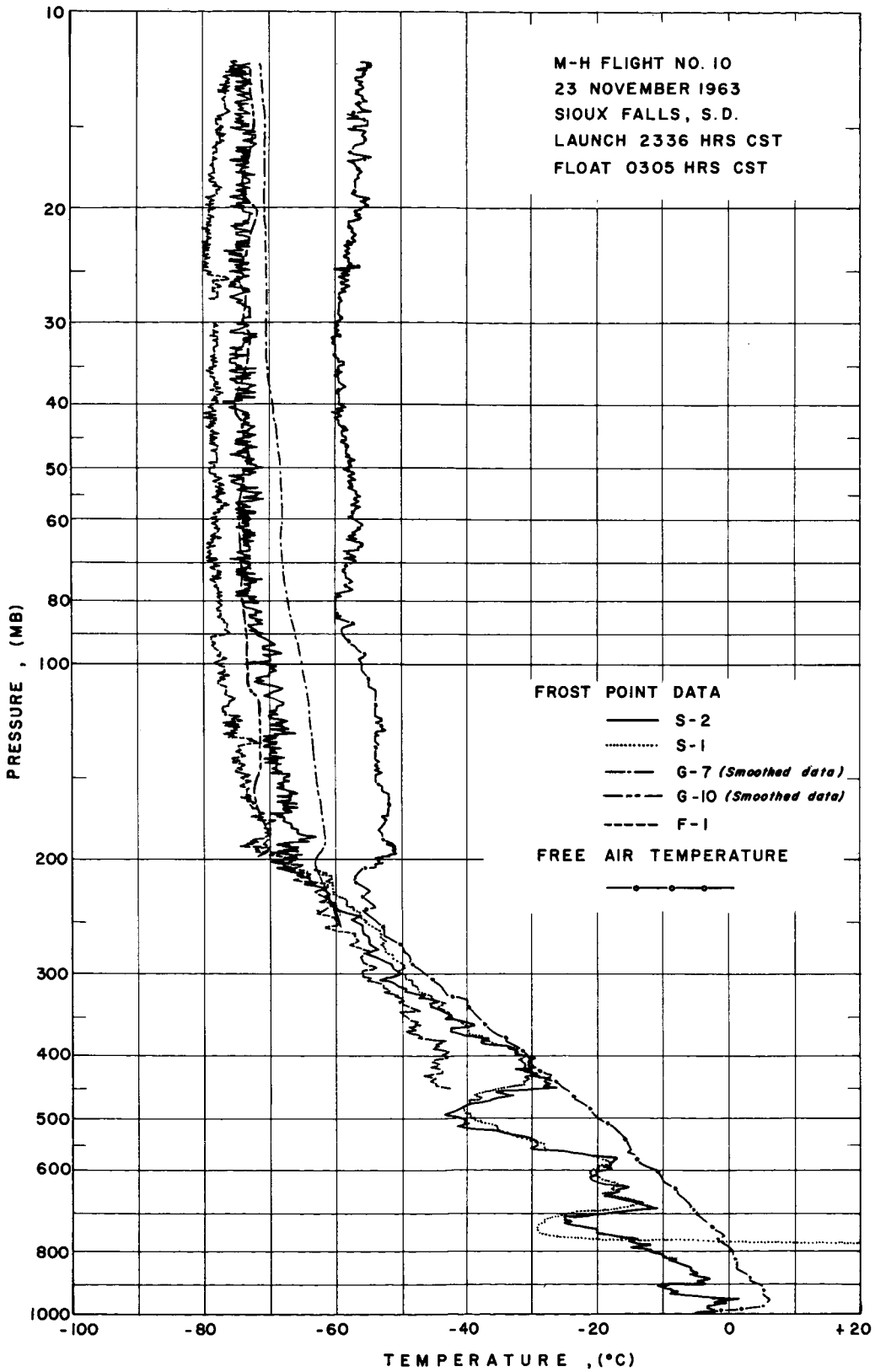

Fig. 4. Ascent frost-point profile, 23 November 1963 flight.

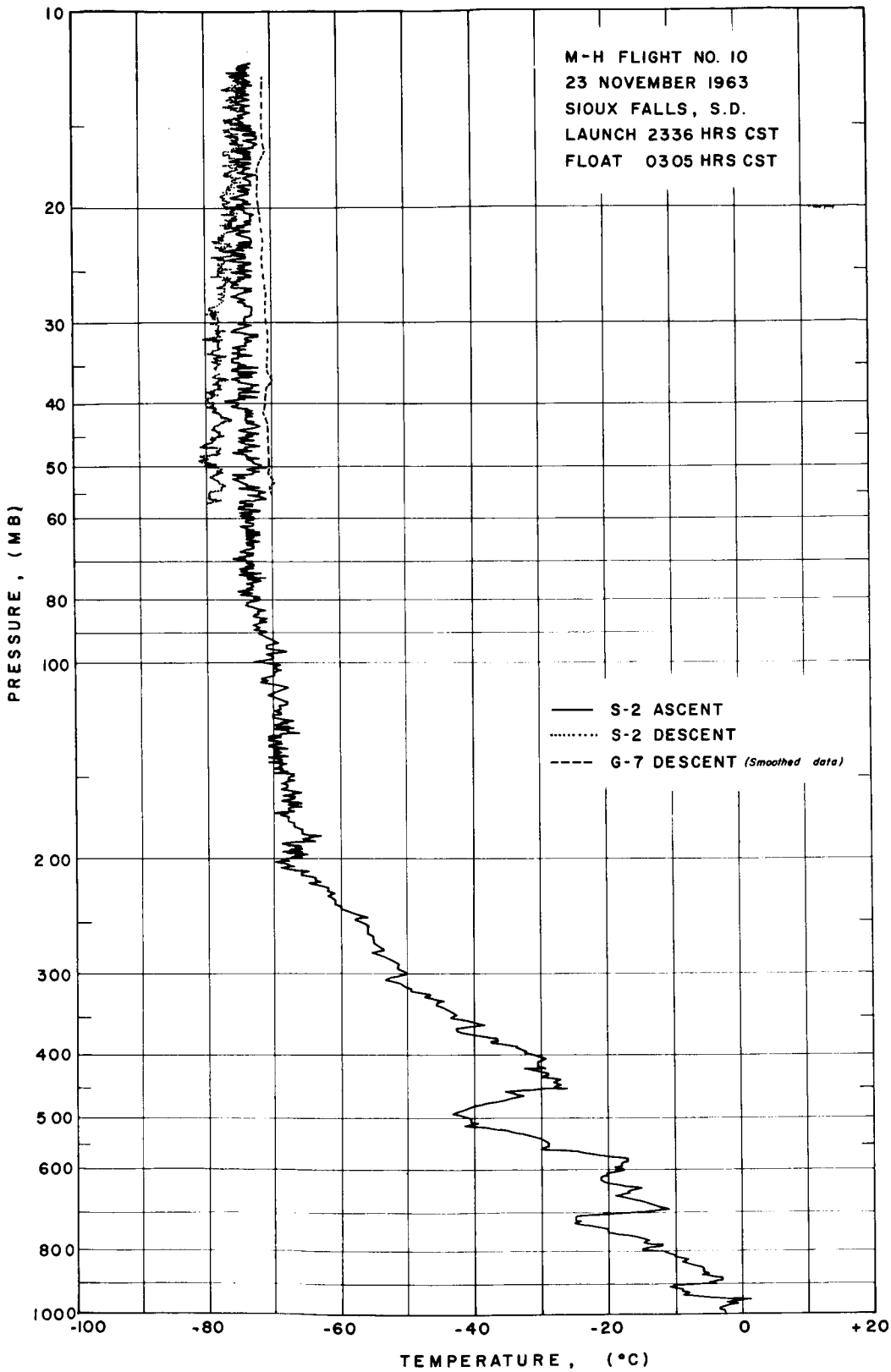

Fig. 5. Descent frost-point profile, 23 November 1963 flight.

V. Conclusions

The frost points measured in two flights by unit S-2 tend to show that there was little change in the frost point above the tropopause in the short time interval between flights. The low frost points found by some observers in this same altitude range were not found here. This does not necessarily imply that these measurements are typical. There may be a great variation in frost point due to difference in latitude, diurnal effects, etc., that can only be established by many and repeated observations. Questionable data caused by instrument malfunction resulting from low mass transfer rates and the errors associated with contamination must first be eliminated. Until such time, the amount and distribution of water vapor in the stratosphere must be considered undecided.

VI. Present & Future Work

At present there are several programs in the field of water-vapor measurement in progress at Honeywell. The purpose of these programs is to determine methods for obtaining reliable frost-point data and to obtain some accurate stratospheric measurements. A study of frost formation, including rates of formation as they apply to individual sensor heads, is under way in a small test cell that can reproduce typical stratospheric conditions in a dynamic fashion.

Work sponsored by the Meteorological Development Laboratory of Air Force Cambridge Research Laboratories is presently being performed to explore the problem of low mass-transfer rates around a frost-formation surface in a rarefied atmosphere. The effect of step changes in frost point on the recovery of the hygrometer and the associated time constants of the hygrometer under various conditions of operation are being studied. The results of these tests will apply to all types of automatic frost-point hygrometers. Preliminary results indicate that with frost points below -70 C and pressures less than 100 mb, time constants of at least 5 minutes should be expected for the response to a varying frost point.

The second program sponsored by the Meteorological Development Laboratory is concerned with constructing a flight package that will reduce contamination as much as possible. Flight tests of this package are included in the program. The amount of contamination will be reduced to as low a level as possible and will be evaluated with the intent of setting limits on the accuracy of the data obtained with this equipment. The program will investigate ascent vs. descent data, night vs. day flights, and other variables such as ascent and descent rates. The package to be developed will be useable on all kinds of flight systems, including long-duration, horizontal flights.

I would like to answer the question raised earlier in the conference about Mr. Mastenbrook's data.

We feel that Mr. Mastenbrook has contributed greatly to stratospheric water-vapor measurements by recognizing the contamination problem. After reviewing some of his data, we are not convinced that his data can be considered as representative of the stratospheric frost-point due to the long instrument time constants that occur at low frost points. It is unlikely that equilibrium over the mirror surface can occur during Mastenbrook's high descent rates due to the low water-vapor concentration that is available for transfer to the mirror surface. Some of his data also show large temperature oscillations, which we believe indicate long time lags in his servo loop. We might not be right either, but the data from our flights through this altitude range appear to verify one another.

Acknowledgments

The authors wish to acknowledge helpful discussions with Mr. M. P. Fricke and the efforts of Messrs. F. Zeeman and C. O'Brien in the flight preparation and reduction of data.

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Nomenclature

Mev	Million electron volts	V	Flip-flop state voltage
N_F	Frost channel long-term-average counting rate	σ_F	Frost thickness
N_R	Reference channel long-term-average counting rate	σ_R	Absorber thickness in reference channel

Discussion

Danielsen — Could you describe the weather pattern or weather situation through which these balloons were released? Were you restricted to a particular type of weather pattern that would bias your sampling?

Rohrbough — We had obtained all the radiosonde data that was available for the days preceding both of these flights, as well as the time in between the flights. During this time in November there were several fast-moving storm systems coming into the area. The upper atmosphere temperatures as indicated by the radiosonde data (they varied at any given altitude by 4 or 5C during a period of 24 - 48 hours) indicated that there was quite a changing stratosphere at this time. We had a snowstorm between the flights of the 17th and 23rd.

Soffen — Do you know how much water there is in the helium? I presume this is a fairly large balloon.

Rohrbough — The amount of water, no. From some of our past work in flying balloons, we have determined that the water vapor concentration in ground level helium has a frost point of about -60C. As one reaches higher altitudes, however, this concentration rapidly decreases below -90C such that the helium should not have much effect. Unless there were exceedingly high diffusion rates from the balloon you would not expect to get much contamination on descent. This is the basis for some observers now taking only descent data.

We did not get large differences between ascent and descent. This was a night flight; it's likely that one might get much more difference on a day flight, due to more water vapor desorption from the balloon and from the package during the day than at night.

Rohrbough — We compared the radiosonde data from the three closest stations to Sioux Falls, which would be Rapid City, St. Cloud, and Omaha. The temperature data varied at different altitudes by about 10C so we felt that we couldn't consider any of those results to be typical of the Sioux Falls area. As a result we used the temperature data that we measured on board our flights.

Steinberg — Let me clarify a point about contamination from the balloon with water. Several years back Goldsmith and associates made a series of experiments putting deuterium into the balloon. They took into consideration the distance they sampled away from the balloon and the amount of contamination they found. If they sampled 100 ft below the balloon they got no contamination, so if one does his sampling at least 100 ft below the balloon he, apparently, will be all right.

Our sample was 150 ft below the balloon.

Junge — What was your distance between the balloon and the instrument involved?

Rohrbough — About 100 ft.

Mantis — I want to point out that the convection currents around the balloon are of the order of hundreds of feet per minute — very much like your descent rate, so that the downwash from your balloon may exceed your downward velocity if you descend 200 fpm at night. You may still have contamination to worry about during the downward part of the flight.

Comment from audience — The daytime flights are free from the downward convection currents so you do not have to worry about balloon-borne contamination then.

Comment from audience — No, I don't think so. Not from the straight lines that he was getting. To get backwash data and attribute them to backwash implies a continual wind moving at the same velocity in the same direction all the time from the balloon toward its sampler. If he had sporadic changes then you might talk about turbulence (in our sampling we'd come across some water and then find it was gone).

Mantis — I didn't mean to suggest that the sample was contamined. I don't know if it was or not. I'm just saying that the currents, the upwash and downwash are of the same order of magnitude as your descent rates. A descent rate of 200 fpm, therefore, does not ensure fresh air.

Rohrbough — On this flight we started descending at 270 fpm and increased slowly to about 300 to 370 fpm.

Large-Scale Distribution of Microorganisms in Atmosphere

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Abstract

Microorganisms in atmosphere can be represented by particles of Stokes' radii of about one micron and larger. Large scale distribution of such particles in troposphere and stratosphere is discussed.

Conclusions for troposphere are based on considerations of physical processes involved and on studies of inorganic aerosols; they seem to be in line with the scanty information for atmospheric microorganisms.

For stratosphere the vertical distribution of particles is calculated for sedimentation-diffusion equilibrium under various assumptions about the eddy diffusion coefficient. It is concluded that even larger microorganisms can penetrate into lower 10 km of the stratosphere. However, an upward penetration of microorganisms beyond 25 km, in extreme cases beyond 30 km, can hardly be expected even for the smallest atmospheric microorganisms.

Exposure of microorganisms to ozone and sulfur oxidation-products in stratosphere is briefly discussed.

I. Introduction

This paper is concerned with the vertical distribution of airborne organisms in the troposphere and stratosphere when these organisms originate at the earth's surface. It is the speaker's intention to survey our present knowledge about atmospheric transport and diffusion of particles in the atmosphere and its application to microbiology particularly in the upper atmosphere. Considerations of this type should form the basis for any interpretation of data on the distribution and origin of microorganisms in the atmosphere.

I shall briefly discuss the following topics:

A. Large scale distribution of aerosols in the troposphere and its implication for tropospheric distribution of microorganisms.

B. Theoretical considerations about the vertical penetration of microorganisms into the stratosphere. The stratosphere extends from the tropopause at about 10 km in middle latitudes to the stratopause at about 50 km. The troposphere normally in our latitudes extends to 11, 12 km and the stratosphere from this level to about 60 km, where it merges with the mesosphere. The level that separates the troposphere and stratosphere at 11 km is called the tropopause.

C. Residence time of microorganisms in the stratosphere and exposure to ozone and oxidation products of sulfur.

II. Size Range of Atmospheric Microorganisms

The large-scale distribution of microorganisms in the troposphere and particularly in the stratosphere will depend upon their fall velocity, i.e., upon their sizes. It is therefore important to consider the size range of the atmospheric microorganisms. Gregory (3) has surveyed the shapes and sizes of a large number of these particles (summarized in Fig. 1). Most of these particles are spores from soil bacteria, fungi, and in addition some pollens most of which have diameters around 20 μ. The sizes given in Figure 1 are diameters, but subsequently all sizes in this paper will be given as radii.

We see from Figure 1 that the diameters cover a wide range starting with 1 μ. Microorganisms originating as they do in most cases from the soil surface, however, occur rarely isolated but rather they occur attached to other inorganic particles, "rafts," so that their effective size for atmospheric transport is enlarged. In addition, smaller atmospheric particles that reside for some time in the troposphere will grow due to a variety of processes inside of and outside of clouds. This results in agglomeration with other airborne particles.

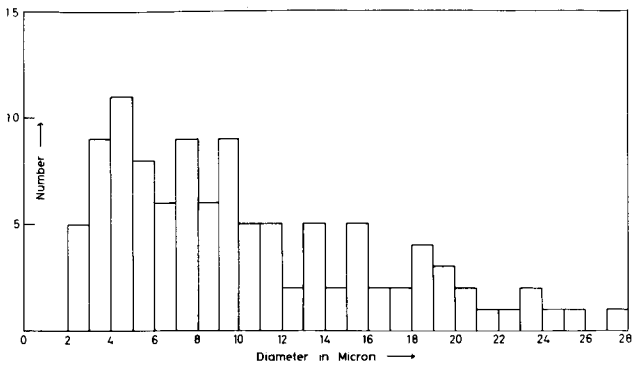

Fig. 1. Number of different types of atmospheric microorganisms as function of their diameter according to compilation by Gregory (3). For non-spherical particles, diameter represents mean value between smallest and largest diameters.

In the course of our further discussion I shall therefore assume a lower limit of about 1 μ radius rather than 1 μ diameter for the atmospheric microorganisms, especially for those that reach the upper troposphere and are attempting to penetrate into the stratosphere.

Besides the size of the particles it is important to know their density. Values in the literature (e.g., Gregory, 3) indicate a range between 0.4 and 1.4 g/cm^3 so that a density of one appears to be a fairly reliable average value. A Stokes' radius of a particle is defined as the radius of a sphere of density one, that has the same fall velocity as the particle itself. We can therefore conclude that the lower limit of airborne microorganisms is a Stokes' radius of 1 μ and that practically all sizes larger than this value can occur.

III. Discussion

A. LARGE-SCALE DISTRIBUTION OF AEROSOLS AND MICROORGANISMS IN TROPOSPHERE. We must admit as meteorologists that we practically know nothing about large-scale distribution of particulate matter in the troposphere. We have only little bits of information that we might tie together to provide a rough answer to this problem. This information may help us, however, to interpret some of the available data, or at least it might serve as a guideline for future work in this area.

The only available data on large-scale distribution of particles, unfortunately, are for particles much smaller than 1 μ. I must admit that the evidence is still rather incomplete. Still, a general picture seems to emerge, from which we can extrapolate to our particular problem. Recent studies of the total number of atmospheric aerosol particles (sometimes called condensation nuclei) seem to indicate that most of the troposphere is filled with an aerosol of 200 to 500 particles/cm^3 (Junge, 4). We do not know the average size of these particles, but we can expect them to have a radius somewhat smaller than 0.1 μ. Their major source is from the continents, especially the populated continents in the northern hemisphere. In continental air near the ground the concentration ranges widely between about 5,000 and 30,000/cm^3. This concentration decreases rapidly with altitude and approaches a background concentration of 200 to 600/cm^3 at about five kilometers altitude (Fig. 2). This concentration stays essentially constant up to the tropopause level. Above 5 km, washout becomes very unimportant simply because the major portion of the rain forms in the lower part of the troposphere, and so washout becomes less efficient. Also, the horizontal wind speeds are normally much larger, so horizontal mixing becomes more efficient. The decrease with altitude in continental surface air is due to various processes; washout and coagulation are the dominant ones.

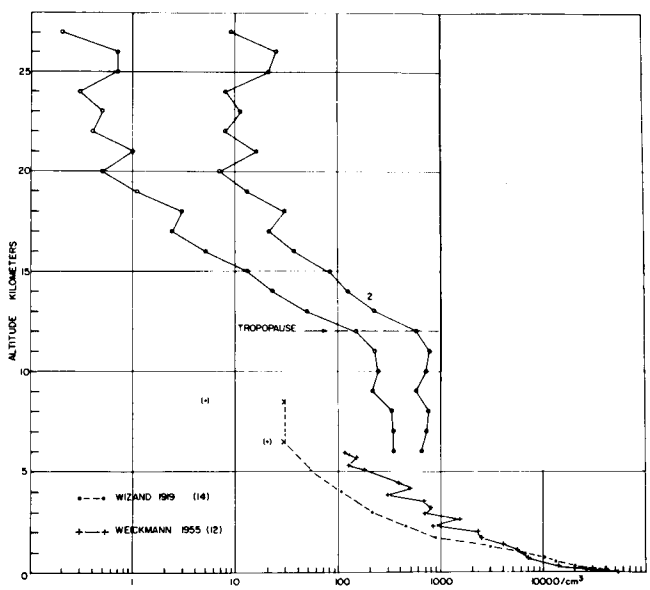

Fig. 2. Average vertical profiles of atmospheric condensation nuclei in troposphere and stratosphere. Measurements in lower troposphere were made by manned balloons (Wigand, 14 flights) and aircraft (Weickmann, 12 flights) over Europe and USA, resp. Profiles 1 and 2 are average of 5 balloon measurements over central USA plotted as concentration (= number of particles/cm^3) and mixing ratio (= number of particles/cm^3 STP) resp. (Junge, 5).

The oceans are practically no source for these particles and it can be expected that the particle concentration remote from continents is more or less uniform throughout the troposphere. This is confirmed by the fact that concentrations of 200 to

$600/cm^3$ are frequently observed at sea level whenever contamination from continental air is excluded.

Studies of atmospheric fission products have shown that this aerosol has an average lifetime in the troposphere of about one month. We also know that it takes air masses on the average about one month to mix horizontally to some extent within the same hemisphere (the mixing across the equator seems to be slower). The fact that hemispheric mixing time and aerosol lifetime are about equal results in a background concentration of aerosols (outside the continental air masses) which usually does not vary more than by a factor of about two in time and space.

All this information pertains to aerosols of less than 0.1 μ average radius; we have practically no knowledge about the behavior of larger particles, that can be applied to microorganisms. There is some evidence that sea-salt particles have a shorter lifetime of about 5 to 10 days. Sea-salt particles form an important though not very numerous category of natural aerosols for which most of the mass is carried by particles between 1 and 10 μ (e.g., Junge, 5) and are thus comparable in size with the air spora. The shorter lifetime is caused by more efficient rainout and washout and by larger fall velocities.

It can be expected that the smaller microorganisms will have tropospheric lifetimes of a similar order once they have left the atmospheric boundary layer. It is reasonable to assume that these shorter residence times will result in larger fluctuations of the background concentration, perhaps by a factor of ten, and also will result in a faster decrease in concentration with altitude over land, i e., over source areas. If we disregard the relatively few microorganisms that originate at the sea surface we must expect that the ocean and also the polar regions act as sinks resulting in a decrease of concentration toward the surface. In Figure 3 are summarized these conclusions and suggestions in a somewhat schematic way.

The rather sparse observations on the horizontal and vertical distribution of microorganisms in the troposphere by and large confirm this picture (Gregory, 3). Living material was present even in such remote places as in the arctic or over the centers of oceans. The concentration in surface air over *land* was in general rather high with rapid decrease with altitude. Over *oceans* there was a tendency for an *increase* in concentration with altitude.

B. PENETRATION OF MICROORGANISMS INTO STRATOSPHERE. There are only a few measurements of the penetration of aerosols from the troposphere into the stratosphere. In Figure 2 are shown these data, which, however, refer to particles smaller than about 0.1 μ . For such small particles the upward flux of particles due to eddy diffusion is compensated primarily by the decrease in number concentration as a result of coagulation, although coagulation does not result in removal of material. Since we do not know for sure if these curves really

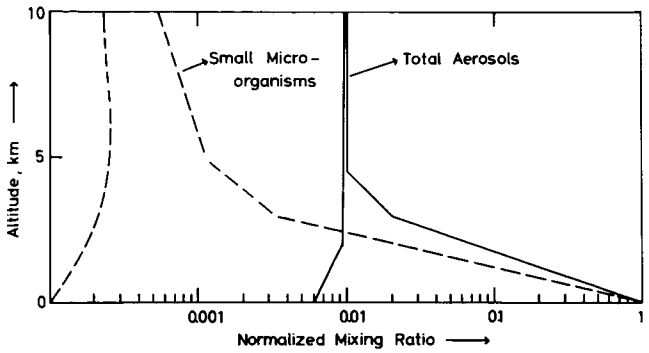

Fig. 3. Schematic diagram of vertical profiles over land and ocean in troposphere: a) Total aerosols *(right)* (condensation nuclei), according to Fig. 2. b) Microorganisms in size range of 1-10 μ radius *(left)* suggested from a) and some information on sea-salt particles. Concentrations are given in relative numbers.

represent a diffusion-coagulation equilibrium and since we do not know the correct size of the particles involved, it is difficult to draw conclusions about the value of D (eddy diffusion coefficient) from Figure 2. For particles larger than 1 μ , sedimentation becomes much more important than coagulation, and for this size range it is therefore likely that we deal with a diffusion-sedimentation equilibrium.

In the following discussion I shall assume that the concentration of microorganisms at tropopause level is uniform. The question now arises how far such particles can penetrate into the stratosphere either by turbulent diffusion or by large-scale, organized circulations. In the stratosphere there is no cloud formation and there is no rain, so washout is eliminated; the only way, therefore, by which particles can be removed is by sedimentation. If we assume for the moment that eddy diffusion is the only responsible mechanism for upward spreading into the stratosphere, we can calculate the vertical distribution for an eddy diffusion-sedimentation equilibrium. The following notations are used:

D = Eddy diffusion coefficient (cm^2/sec)
H = Scale height of stratosphere (6.4×10^5 cm)
n = $n_0 e^{-z/H}$ number density of air molecules (cm^{-3})
r = Particle radius (cm)
w = Fall velocity according to the well-known Stokes-Cunningham formula which gives w as a function of r and the mean free path length of the air molecules. (cm/sec)
z = Altitude above tropopause (cm)
ν = Mixing ratio of particle number to air molecules

The *concentration* of particles is $n \cdot \nu$; the downward flux of particles due to sedimentation is $n \cdot \nu \cdot w$, and the upward flux due to eddy diffusion

and the gradient of ν is $D \cdot n \frac{\partial \nu}{\partial z}$

For equilibrium we have $\nu w + D \frac{\partial \nu}{\partial z} = 0$

which gives the solution

$$\nu = \nu_0 \cdot \ell^{-\int_0^z \frac{w}{D}\, dz}$$

where ν^0 is the mixing ratio at tropopause level. Reliable evaluation of this expression is difficult because of our lack of information on D and its variation with altitude. In Figure 4 are summarized some of the measurements or estimates of D; this shows the degree of uncertainty to be about one order of magnitude or even more. It is certain that D is markedly lower in the stratosphere than in the troposphere due to the higher stability within the former and it is certain that D decreases with increasing distance above the tropopause. In order to cover the range of possibilities indicated in Figure 4

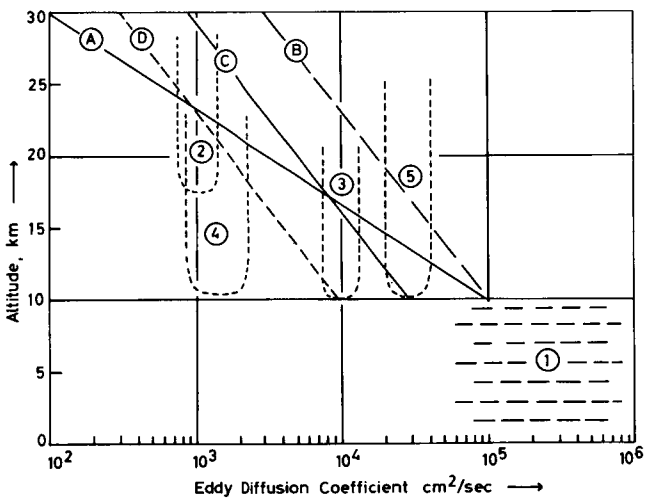

Fig. 4. Eddy diffusion coefficient in troposphere and stratosphere. 1) Approximate range in the troposphere (see e.g., Lettau, 7). 2) Tropical stratosphere from fission product observations (Spar, 8). 3) Same as 2), but for polar stratosphere. 4) Estimated value by Brewer (1). 5) Estimated value for stratospheric sulfur layer (Junge, 6). For our calculations we approximated these values by four models. Models A and C most likely represent average conditions, models B and D, extreme conditions.

I assumed four models of the vertical profiles of D. Models A and C, I feel, are closer to the actual average values whereas models B and D represent extreme values.

In Figures 5 and 6 are the results of the computation of ν. The *actual concentration* of particles per cubic centimeter is given by the product, $n \cdot \nu$; n decreases with altitude with a scale height of 6.4 km, so that the decrease amounts to 0.1 for z = 14.4 km. This should be kept in mind for the discussion of the ν values. For models A and C, particles with a Stokes' radius of 1 μ can hardly penetrate beyond 25 km, but particles between 1 and 10 μ radius can well spread into the lower stratosphere. For the extreme models B and D the altitude to which 1 μ particles can penetrate varies between about 19 and 28 km.

On the basis of these calculations one should expect that in middle latitudes with a tropopause height around 10 km no microorganisms would be found above about 25 km. This limit will probably also hold for tropical latitudes, since here the tropopause is about 6 km higher, but also D is definitely smaller, so that both effects will cancel each other more or less.

Unfortunately it is not entirely certain if eddy diffusion is the only way of transport in the stratosphere. Several models of an organized circulation have been suggested in the literature of which the Brewer-Dobson model is the best known (see e.g., Junge, 5). But recent studies of fission product distributions within the stratosphere make it rather certain that the role of this circulation, if it exists at all, is of minor importance. There is firm evidence, however, that in late winter, e.g., in January and February, there occur large scale vertical motions in the polar winter stratosphere, that result in sudden dynamic warmings of the layers between 15 and 30 km, but extend perhaps as high as 50 to 60 km. Craig (2), e.g., found large areas with *subsiding* motions of up to 5 cm/second that continued for several days. It is not unlikely that *upward* currents of an equal magnitude occur simultaneously, that should be able to carry particles smaller than a certain limit along with them to rather high altitudes.

The figures in Table I give the radius of particles which have a vertical velocity of 5 cm/second.

With our assumption that the lower limit of atmospheric microorganisms is a Stokes' radius of 1 μ we conclude that vertical motion associated with these sudden warmings can hardly carry living material beyond 30 km. From our present knowledge of stratospheric behavior, which is admittedly still rather scanty, 30 km thus seems to be the upper limit to which microorganisms originating from the earth can penetrate.

C. RESIDENCE TIME OF PARTICLES IN STRATOSPHERE AND EXPOSURE TO OZONE AND OXIDATION PRODUCTS OF SULFUR. I shall conclude this paper with some remarks about the average time microorganisms can be expected to remain in the stratosphere before they are removed again by sedimentation. If

$$N = \int_z^\infty n \nu \, dz \; (cm^{-2})$$

Table I

Particle Size for Fall Velocity 5 cm/sec

Altitude, km	15	20	25	30	40
Particle radius, μ	2.5	2.2	1.6	1.0	0.3

is the total number of particles per square centimeter of the earth's surface above altitude z, and $(n \nu w)_z$ the sedimentation flux at z ($cm^{-2} sec^{-1}$), the average residence time, t, is

$$t = \int_z^\infty n \nu \, dz / (n \nu w)_z \text{ (sec)}$$

The following figures have been calculated for Model C with $D_o = 3 \times 10^{-4} cm^2/second$.

From fission product studies we know that the general residence time for air in the stratosphere is about half a year below 15 km and about one year below 20 km. This is therefore an upper limit for all particle sizes. The figures in Table II refer to diffusion-sedimentation equilibrium only and should be replaced by these general figures in those cases in Table II where they are larger, i.e., for radius 3×10^{-5} cm below 20 km. If we assume Model C to be representative for average conditions we can conclude that small microorganisms with radii around 1 μ will reside about two months above 15 km and about two weeks above 20 km. These times should be considered as rough estimates, but may be of importance with respect to the exposure to ozone. The average ozone concentration in middle latitudes, in spring and fall can be given by the figures in Table III.

Besides ozone there is another unfavorable aspect to survival at these altitudes that was only recently discovered. Studies indicated the existence of aerosols between about 15 and 25 km that consist primarily of sulfate (see Junge, 5). It is not known if this sulfate is free or if it is neutralized by some cation. It is likely that these particles represent the product of photooxidation of SO_2 that penetrates as

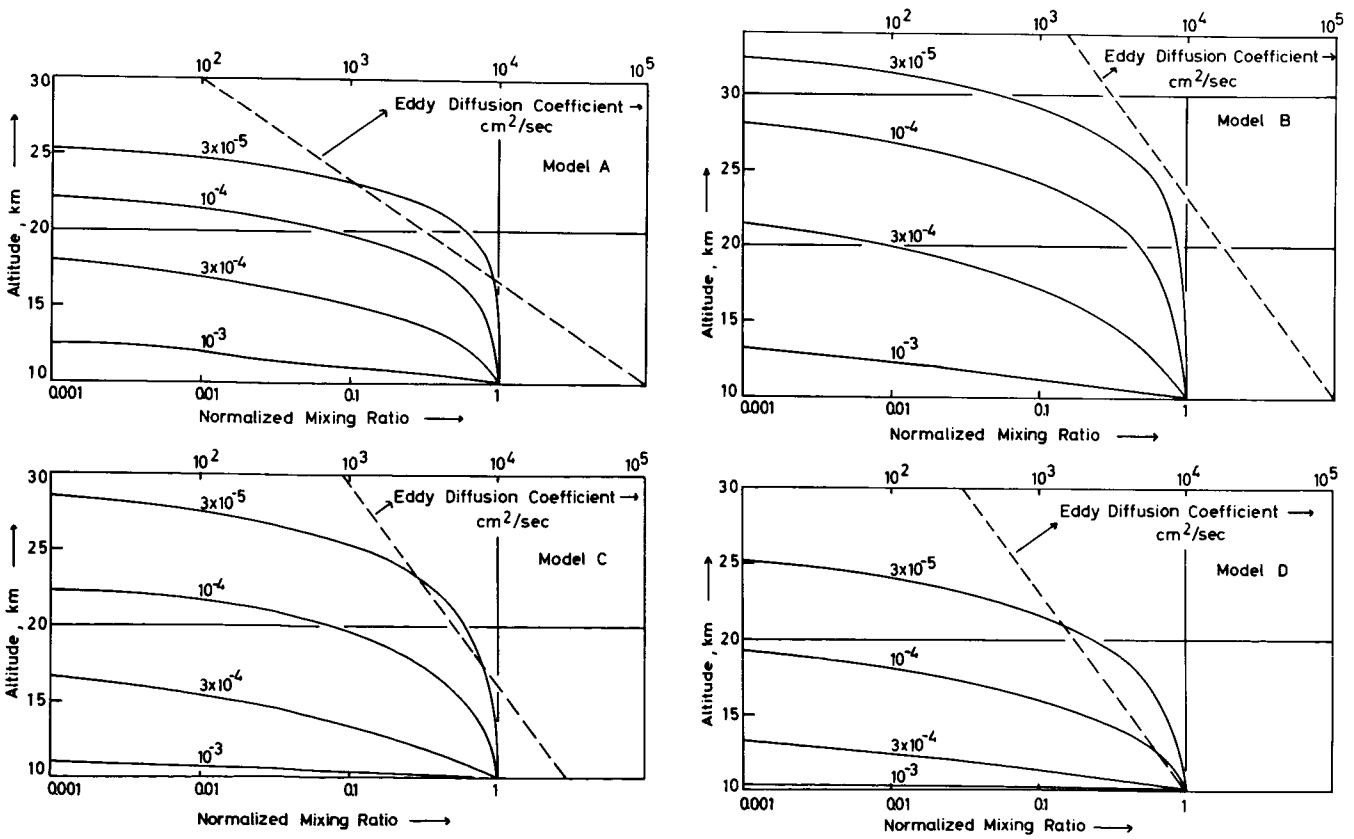

Fig. 5 *(left)*. Calculated vertical distributions in stratosphere for particles of various Stokes' radii for models A and C.
Fig. 6 *(right)*. Same as 3, but for models B and D.

Table II

Residence Time in Years Above Altitude z* for Particles of Various Stokes' Radii r

z* =	10	15	20	25
$r = 3 \times 10^{-5}$ cm	5.60 yr	2.85 yr	0.97 yr	0.17 yr
$r = 10^{-4}$ cm	0.56 yr	0.25 yr	0.043 yr	...
$r = 3 \times 10^{-4}$ cm	0.025 yr	...	...	...

*Altitude in km above tropopause.

gas from the troposphere where it is always present, into the stratosphere. If this concept is correct, one should expect that these particles consist primarily of concentrated sulfuric acid and that every particle present in the stratosphere, e.g., also microorganisms will act as a condensation nucleus for SO_3 in the presence of water, of H_2SO_4. The amount of H_2SO_4 that can condense on a *single* microorganism may be as high as 10^{-6} μ g. This amount corresponds to a mass equal to that of the smallest spores themselves and should be crucial for their survival.

This latter discussion has been restricted to these two dangers, i.e., ozone and sulfates, to survival by *constituents* of the air, because those dangers of extreme temperature, dryness, and shortwave radiation are already often discussed and need no further emphasis. Altogether both the chemical and the physical dangers will shorten the lifetime of microorganisms in the stratosphere in one way or another, it is possible that the limitations by transport mechanisms for vertical spreading within the stratosphere are exceeded by the limitations imposed by the possibilities of survival.

Table III

Average Ozone Concentrations in Spring and Fall in Stratosphere in Middle Latitudes

	Altitude, km		
	15	20	25
Ozone concn., μ g/m^3			
Spring	210	280	260
Fall	110	170	210

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Discussion

Gregory — First of all, in appreciation of this paper, one small correction for which I am responsible, perhaps. The size we should consider is probably down to about half a micron. Some bacteria are smaller than that, but we don't know any mechanism by which they could get into the air as single cells.

I would like to ask one question. It is sometimes assumed, perhaps incorrectly, that under certain conditions microbes in the air do not merely lose viability; they lose visibility also. They can as it were, dissolve. Is there any evidence for that? Their envelope is probably a carbohydrate on a protein shell; inside is mainly protein. These substances are resistant except to high temperatures, combustion, and enzymatic action. What would happen to such a particle in the stratosphere with a coating of sulfuric acid on it when acted on by ozone? It might simply disappear.

Junge — This is a question which you would rather ask a biologist than a meteorologist. I don't know if a microorganism can survive for a time after you put a little bit of sulfuric acid on it. We have no information from our discipline which could answer both your questions.

Goetz — Is it not rather also a question of the temperature at which this contact occurs when the thermal energy level as such is quite low? It is thinkable that ozone in the tropopause may not interact, simply due to lack of "kT" — if I may put it that way. There are some experiments available, where the persistence of bacterial aerosols at low temperatures was tested. Destruction at this kT level, if any, was much smaller than at the 300 K level.

Goetz — On the other hand the organisms when airborne seem extremely sensitive to photons. Maybe some photochemical interaction occurs in the cell even at low temperatures. The $h\nu$ probably can do a little bit without the kT. That evidence seems to be available. The irradiation, of course, also will be substantial in the ultraviolet at the altitudes which you describe. It is questionable whether or not ultraviolet can be more destructive than the chemical interaction with sulfuric acid and ozone at the thermal wall of the biosphere.

Junge — This is right. I didn't mention the irradiation simply because I found that this is well discussed in biological papers. I wanted only to mention those effects which might have been (well, not in the case of ozone, but in the case of sulfuric acid) overlooked or unknown to those who are interested in these problems.

Church — With regard to H_2SO_4 or SO_2. Consider the Thiobacillus, for example, that lives quite well at 1 N sulfuric acid and utilizes the sulfate radicle. Conceivably, there are sulfate reducers that could get into these upper levels. All of these compounds discussed and considered to the chemodisinfecting materials are restricted to and selective for certain organisms. In the soil, for example, there is a tremendous variety of microorganisms which may take some of the so-called disinfecting materials and use them as carbon-energy sources for growth. Although we worry about microorganisms disappearing or losing viability in outer space, we should also consider that certain of these gases and organic materials may actually induce altered metabolic systems in the organism. Some of these phenomena are now coming to light in studies of terrestrial organisms. Meteorologists and physicists who are having problems with studying the microbial ecology of outer space should not become disillusioned or discouraged because biologists are just beginning to learn a few things about microorganisms that we've been living with all our lives.

Dimmick — I have three questions, one out of ignorance. Is a bacterium at your 30-km height high enough so that photon pressure can cause this organism to migrate farther?

Junge — No.

Dimmick — The second question is this: If we want to simulate this condition in the laboratory, would we need extremely sophisticated equipment?

Junge — No, you would not need sophisticated environmental conditions.

Dimmick — What pressures are involved?

Junge — The pressures involved are on the order of 10 mb.

Dimmick — Meteorologists talk about millibars and botanists talk about microns in pressure... then in millimeters. Yes, that's simple enough. [Editor's note: 1 bar equals 10^6 dynes cm^{-2}. Since 1 atm is the pressure of 1.01361×10^6 dynes cm^{-2} one bar is 0.98962 atm. Since 1 atm equals 760 mm Hg, 1 mb is roughly equivalent to 3/4 mm Hg.]

Junge — I would say it is not particularly difficult to simulate these conditions, even so far as radiation and ozone are concerned.

Halvorson — If all you know about these is their effect on the organism when there is plenty of water present, it is dangerous to postulate what toxic agents may do to organisms in the atmos-

phere. When organisms are completely dehydrated the effect of these agents would be quite different than when they are not dehydrated. I should think that when completely dry, a bacterial spore would not be bothered by ozone.

Tsuchiya — The discussion until now has been about viable organisms. All discussions at this particular conference have been directed toward the general area of exobiology. How should the NASA people reason if they were to get non-viable organisms (if they could develop methods for detecting organic matter up there)? I'm addressing this not only to Dr. Junge, but to the entire group here.

Junge — One micron was suggested as a lower limit for particle size. This limit would shift perhaps to half a micron. In the face of our present meteorological information, I would say that 30 km or something of that nature should probably be the upper limit at which one can detect terrestrial microorganisms dead or alive. If one finds something beyond that altitude I would say from my own reaction here that I would be suspicious; it might have another origin.

Phillips — Dr. Tsuchiya, I think every one of us expects that anywhere we might find viable microorganisms we would find much larger numbers of the same types of organisms that have died, because we know there's a constant death rate for microorganisms. The rate is different for different types of organisms, for different temperatures, for different relative humidities, but one cannot get a number of viable organisms without getting a considerably larger number of non-viable organisms.

Our problem is that a viable microorganism can be counted easily because it will multiply and produce quite a lot of matter within 24 hours; a non-viable organism is a question of rather innocuous chemical material which weighs about 10^{-12} g. This poses quite an analytical problem on detecting non-viable organisms.

Tsuchiya — Dr. Phillips, you do not foresee a situation, then, where one would get all non-viable material, or let us just say organic material without viable organisms?

Phillips — It is quite likely that one would probably in the places mentioned. I was about to thoroughly agree with Dr. Goetz, for example. We're talking about ozone, and so on — some toxic materials. The same source of energy, namely radiation, that causes oxygen to go to ozone acts directly on organisms and causes lethal chemical change within the microorganisms themselves. Quite likely at those altitudes one would find nothing but dead material. The higher one goes the higher the ratio of dead to living cells. The organisms have been in the air longer, obviously. The higher they get the more they are exposed to radiation, etc.

Tsuchiya — In terms of exobiology, now, if you are told that organic matter is on your probe, how are you to interpret these data? Is the matter coming from our planet or elsewhere?

Phillips — One of the first thoughts is that the organic matter might be some kind of air contamination. But samples can be enclosed in the sampler, can be brought back uncontaminated by their trip through our atmosphere. Unless precautions are taken against contamination, any exobiological experiment is open to doubt.

MacLeod — To leave exobiology just for a moment, Dr. Junge, there have been some missions to collect particles in the noctilucent cloud layers (which are approximately 80 or 90 km) and also missions to collect particles in the region of 160 to 170 km aloft. There is some evidence that particles may well have been collected in both of these regions. Are the dynamic mechanisms to which you referred earlier, similar mechanisms? Top limits would be at 30 km. By the way, the particles are, I think, in the size range between approximately 0.1 μ and 1 μ.

Junge — Mechanisms which may bring these other particles way up?

MacLeod — The methods which you were talking about, which would put a top elevation of 30 km for terrestrial organismic distributions.

Junge — No, I think these other particles collected at 80 km or 160 may come in from outer space. If I understand your question correctly, there is no upper limit for those particles.

MacLeod — You would conclude that according to the dynamic models that you have postulated here, if you did get organic materials from the 70 to 170 km region they should be from outside?

Junge — It should be from outer space, exactly. I estimated this 30 km limit all around as a kind of working figure. It may not be too correct at the moment, but at least it may give roughly the order of magnitude. From my diagrams you can see that changing the parameters over quite a range really doesn't affect this upper limit too much. So even if you put another 10 km on top of 30 km, that is, at 40 km, in my eyes this would positively be an upper limit beyond which particles of 1 μ radius can practically not penetrate. I would say this is a rather safe statement.

Srinivasan — I think Dr. Tsuchiya has a point there as far as exobiology is concerned, because in all the sampling equipment we have, we just keep the equipment, track it somewhere, and then bring it down. We know that there are some anaerobic terrestrial organisms which when exposed to oxygen become non-viable. In the same way it is highly probable that the type of environment in which they are viable may alter in such a drastic way that the organisms might lose their viability by the time we bring them down to the earth and then analyze them.

Brown — I'd like to be reassured as to what the numbers of the calculation are. It's been established, I believe, that if we locate organic matter at these very, very high altitudes, the assumption is that the matter was obtained either by contamination of the sampler or from outer space. If some of this organic matter turns out to be viable, the same implications are involved. But there is also the possibility of calculating how long organic matter

could have existed in that environment and still remain viable. Aside from the hazards of the sulfuric acid, and so on, the radiation flux is probably the most damaging environmental influence. I assume that the microbiologists know for dry organisms at high vacuums or moderately high vacuums, how long to expect these organisms to remain viable. Do we have a number in units of time, that says that if there are viable organisms that are returned from samples taken at 40 or 50 km they could not have been there longer than 8 minutes, 6 years — what is it?

Bruch — Dr. Gerald Silverman has a NASA-sponsored study to determine the resistance of microorganisms to ultraviolet fluxes under ultra-high vacuum (10^{-9} torr). He has achieved rapid kills of bacterial and fungal spores on smooth surfaces. When he started working with millipore filters, he found that the surface of the millipore filter afforded enough protection for some of the organisms to resist a high ultraviolet flux. The problem that Mr. MacLeod brought up is a significant one: if there is any particulate matter in space and if the microorganism can attach itself in a way that it is shielded from this high ultraviolet flux, then that organism will resist destruction by ultraviolet.

Brown — You mean that sulfuric acid may protect the organism?

Bruch — Yes, if it absorbs ultraviolet.

Oswald — Dr. Junge, I believe you gave us some data that we want. You said 260 μ g of ozone per cubic meter of space at 25 km. This leads to some interesting calculations because this would be about 10^{17} molecules of ozone. Of course, in a cubic meter there are about 10^{18} cubic microns. This would mean that we'd have about one ozone molecule per cubic micron of space. Our hypothetical bacterium occupies about one cubic micron. This would indicate to me that the chances of an encounter between ozone and a bacterium would be rare and chances of ozone kill of bacteria at that elevation would therefore seem slight.

Junge — This is a biological question. I have no idea on that.

Bruch — I can't address myself completely to the question of water. I would prefer that Dr. Halvorson or Dr. Church discuss water relationship in spores. From my experience with the destruction of spores, it's very difficult to kill those which are dry. Others may disagree with me.

MacLeod — I would like to agree with Dr. Greene that we face a serious problem in drawing any conclusions from the return of organic matter from high elevations. This is probably THE most serious problem. Right now we do not have methods sufficiently sensitive to accurately measure material from this kind of exploration, or methods by which we can get a large enough sample to answer our questions. We cannot determine if material is dead or alive; we cannot determine if we are interpreting in terms of our own upper altitude, the moon, Mars, or what, and — extremely important — we do not know if we are getting false signals from contamination.

Biological Significance of Bacterial Counts in Aquatic Environments[1]

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Abstract

23991

Abundance of bacteria in natural environments, as determined by direct and indirect techniques, is often implicitly regarded as an estimate of microbial activity in general. Some recent marine bacteriological studies are discussed in detail with regard to adequacy of techniques and with regard to possibility of an ecologically significant interpretation of counts obtained. It is concluded that to obtain statistically significant counts of any specific metabolic type of bacterium presupposes knowledge of growth requirements, germination properties, and extent of clumping and overgrowth. While under such conditions bacterial counts represent fair estimates of concentration of these organisms (regardless of their physiological state), they are not acceptable as an adequate measure of bacterial activity in natural populations. New approaches, employing continuous and semi-continuous growth systems, are promising more realistic estimates of bacterial activity *in vivo*.

Author

Counting of microorganisms certainly is not a spectacular topic to talk about, at least for microbiologists, and yet, considering the universal use of this technique, it is surprising how rarely its intrinsic biological significance has been examined. Judged from experience of the aquatic microbiologist, the request to discuss this particular problem at the present conference seems to be well justified.

In descriptive biology, the abundance of an organism in a certain habitat is the criterion generally used to estimate the degree of its adaptation to this particular environment. Unfortunately, this general concept is not applicable in the ecology of *microorganisms* in water, soil, and air. The various difficulties of relating bacterial counts obtained by classical techniques to the total number and overall activity of the bacterial population has been realized and discussed from time to time. The lack of adequate bacteriological techniques to study natural substrates, however, still is resulting in the accumulation of data of little significance.

In the atmosphere, the mere abundance of bacteria is of great interest. No considerable metabolic activity can be expected. Counts of bacteria in water and soil, however, always imply an estimate of their overall activity. This fact includes inherent difficulties of technical and biological nature.

The techniques for counting bacteria in water consist of a large variety of procedures, which can be condensed to two main methods: 1) the *direct* microscopic observation of dried and stained samples on slides or membrane filters, and 2) the *indirect* means of counting colonies of bacteria on or in solidified agar media after inoculation with the sample and incubation for a certain period. In the latter technique it is presupposed that each individual cell of the sample will develop to a visible colony.

The microscopic technique was first used intensively by Winogradski about 80 years ago [re-edited by Winogradski in 1949 (5)]. The cultural technique was first described by Koch in 1881 (3). Koch in his original publication did not suggest the application of his technique for enumerating bacteria in mixed or even in natural populations. He referred to populations of bacteria with uniform growth requirements where 90 to 100% of the viable cells actually can be recovered. From general experience in aquatic environments, only 1.0 to 10% of the bacteria present can be detected by enumeration of grown colonies; the percentage varies with the type of water investigated. Similar figures are encountered when applying the direct microscopic technique. Here errors arise from the difficulty of differentiating bacterial cells from particulate organic matter and the inability to distinguish living (viable) from non-living cells. There is no reason to believe that the percentage of inactive cells is constant or usually low.

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From the methodical point of view, microorganisms differ from higher organisms basically by their small size, their ability to form highly resistant stages, and their metabolic versatility. As a corollary of these properties, (excellently dealt with from the ecological point of view by van Niel and Stanier, 4) most bacteria are ubiquitous, with the few exceptions of pathogenic and highly specialized forms. Their presence does not necessarily indicate any metabolic activity. On the other hand, one might not detect species that are active in the habitat because they do not grow on the media used.

The term "total count" is misleading as far as complex natural populations are concerned. Counting a specific metabolic type as a partial population is not only more accurate from the technical point of view, but also is more indicative of the characterization of the habitat.

It must be kept in mind, of course, that growth of a species on a selective medium does not indicate that the same metabolic action takes place in the natural environment. Most protein-decomposing bacteria that grow on a peptone medium, for instance, can grow at the expense of organic acids in the natural habitat as well. Some *Pseudomonas* types are able to satisfy their energy and carbon requirements by oxidizing any one of approximately 100 simple organic compounds.

In general, it must be emphasized that the total activity of the bacterial population is not indicated by the size of a partial population, as often has been assumed. In addition, opposing microbial reactions in mixed cultures can result in high numbers of bacteria, but in no detectable biochemical activity. These problems have to be considered to get the most realistic information from bacterial counts.

In Table I, direct and indirect counts are compared in different types of water. A medium containing peptone and yeast extract was used in all cultural techniques. A relatively low colony count can be caused by a low percentage of proteolytic forms within the microbial population and by the tendency of many bacteria to form aggregations under natural conditions. One colony can grow from a single cell as well as from a clump of hundreds. Microscopic counting has yielded high bacterial numbers in waters rich in nutrients, but has yielded comparatively low numbers in impoverished waters.

A more elaborate study was made to collect information on the bacterial population of offshore seawater (2). Five samples were taken from the surface to a depth of 200 meters and processed by six different counting techniques (Table II). After filtration of the sample and transfer to agar medium, colonies were grown on membrane filters (MF) (macro colonies on MF, see Table II). By this technique, only insignificantly more bacteria were found as compared with the ordinary cultural technique (plate count). The serial dilution technique ("most probable number") and the micro-colony technique gave considerably higher counts. The latter method involved counting of colonies after the first stages of development (3-6 hr of incubation instead of the usual 48 hr). By this means overgrowth of fast-growing colonies over others could be observed and considered. Also in this technique, a decreased nutrient concentration and lower temperature of incubation were supposed to favor slow-growing marine bacteria. The direct microscopic counting (direct counts on MF) (see Table II) was greatly improved when the concentrate of the sample was transferred from the membrane filter to slides (Cholodny technique).

More striking than the numerical differences between direct and indirect counts is the fact that apparently different groups of bacteria are counted by each of the techniques used. Particular morphological types observed in the microscopic preparations could not be recovered on agar plates. For example, a stalked bacterium (*Caulobacter*) was abundant in a sample taken at 75 meters but did not grow on any of the agar plates inoculated with

Table I

Indirect and Direct Bacterial Counts in Various Aquatic Environments
(from Jannasch, 1)

	River				Sea	
	Rather clean section	Highly contaminated section	Eutrophic lake		Harbor	Offshore sea
Total depth at station, m	1.8	2.1	3.8		6.0	1,200
Depth of sampling, m	0.5	0.5	0.1	3.7	1.0	1.0
Bacteria as 10^3/ml						
Plate counts	77.0	826.0	1.1	143.0	6,820.0	0.341
Direct counts	289.0	11,230.0	0.52	2,600.0	83,270.0	0.163
Micro-col.	11.7	0	42.0	1.3	0.06	0.280

Table II

Ratio of Microbial Counts, Range of Values, & Percentage Error of 5 Different Techniques Compared to Plate Counts*

	Plate counts	Macro-colonies on MF**	Serial dilution	Micro-colonies on MF**	Direct counts on MF**	Cholodny techn.
Mean	1	1.16	21.8	32.1	147	2,100
Range	...	0.2-4.0	0.3-35	1.6-34	13-840	108-9,700
Error, %	...	17.1	19.2	12.7	8.5	20.7

*Counts were obtained from 6 samples (surface, 25, 50, 75, 100, & 200 m) of offshore seawater. (from Jannasch & Jones, 2)
**Membrane filters

the same sample. None of the chemosynthetic and photosynthetic bacteria developed on most media used for counting purposes. Little is known about abundance and quantitative activity of these organisms although their significance to the cycle of matter is evident. The further elaboration and application of counting techniques for characteristic types of bacteria is more important and more indicative than "total counts."

The development of new methods in microbial ecology has also to consider the dynamic aspects of bacterial growth response to environmental conditions. From this point of view recent experimental application of continuous and semi-continuous growth systems seems to be most promising. Still, taking bacterial counts will be a valuable tool as long as the results undergo appropriate interpretation.

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Discussion

Phillips — No one can ever get an absolute total count of any group of mixed microorganisms. I thoroughly agree.

I do think you picked out some horrible examples in order to point out the problem, that is, you chose some of the worst conditions that one could have selected in getting a total count in mixed cultures.

Were these samples from various natural waters? Did you shake them? With a detergent? For how long?

Jannasch — We shook the sample with Tween 80 for about 3 to 4 hours on the shaker; still the clumps did not break up completely. There was a slight increase in numbers, about twofold, but this was all.

Phillips — This was your usual technique for breaking up clumps? It ordinarily works?

Jannasch — Yes, as far as it seemed useful to us.

Goetz — On this technique that you just described, were the cultures still active when the pictures were taken? [Note added by Jannasch in proof: Micro-photographs shown during the talk were taken from (2) and from (1): H. W. Jannasch. 1958. J. Gen. Microbiol. 18: 609-620.]

Jannasch — You mean the colonies on the membrane filters? Oh no. They were fixed with formalin and stained.

Goetz — About 15 years ago, when at Caltech we developed (A, B, C, D) the membrane filters and their microbiological applications for the Chemical Corps in close collaboration with Dr. Phillips, we came across a technique by which one can observe the growth of organisms microscopically without interfering with their activity.

Jannasch — This can be done on several surfaces such as membrane filters, agar films, and on other solid media.

Goetz — I brought along two slides from that period because the indications are similar to yours and support the views which have been presented.

The technique we developed consisted of producing black membranes with a particularly smooth surface in order to minimize diffuse light scattering under the microscope by reflected low-angle dark-field illumination [(mirror-condenser-Ultropak system) Leitz]. The bacteria and other particles retained by filtration of air or water on the membrane surface become visible as discrete scattering objects even under relatively low magnification. When the membrane was contacted from below with a nutrient while under the microscope (with somewhat elaborate precautions to maintain constant temperature and humidity levels), the growth and subsequent colony formation of the live particles changed their scattering pattern while that of the neutral particles remained the same.

In Figure A is illustrated this growth process at exactly the same site (ca. 4 x 10^{-2} cm^2) of a membrane culture (*E. coli*) after 4, 6, and 7 hours incubation at 37 C with about 50 x linear magnification.

In Figure B are shown the same photos as superimposed pairs of the 4 to 6 hours (*left*) and the 6 to 7 hours (*right*) cultures. As they result in a perfect fit, one can follow the growth pattern of almost every colony. As an example, the "giant" complex at the lower right in 2 hours extended its area about 14-fold (3 x 10^{-5} - 4.6 x 10^{-4} cm^2); in the next hour it expanded but two times. The smaller grow accordingly faster at the later stage. The tendency of adjacent small complexes to coalesce into larger units is quite obvious as well.

Jannasch — Are you sure there are microorganisms in there, that there is growth?

Goetz — From the optical viewpoint this system has the advantage of being much less limited in resolving power than the customary optics, and the biological preference for this type of dark field over that of transmitted illumination is due to the fact that the organisms do not have to absorb, but only to reflect or scatter the illuminating radiation for producing the optical signal, namely, their image.

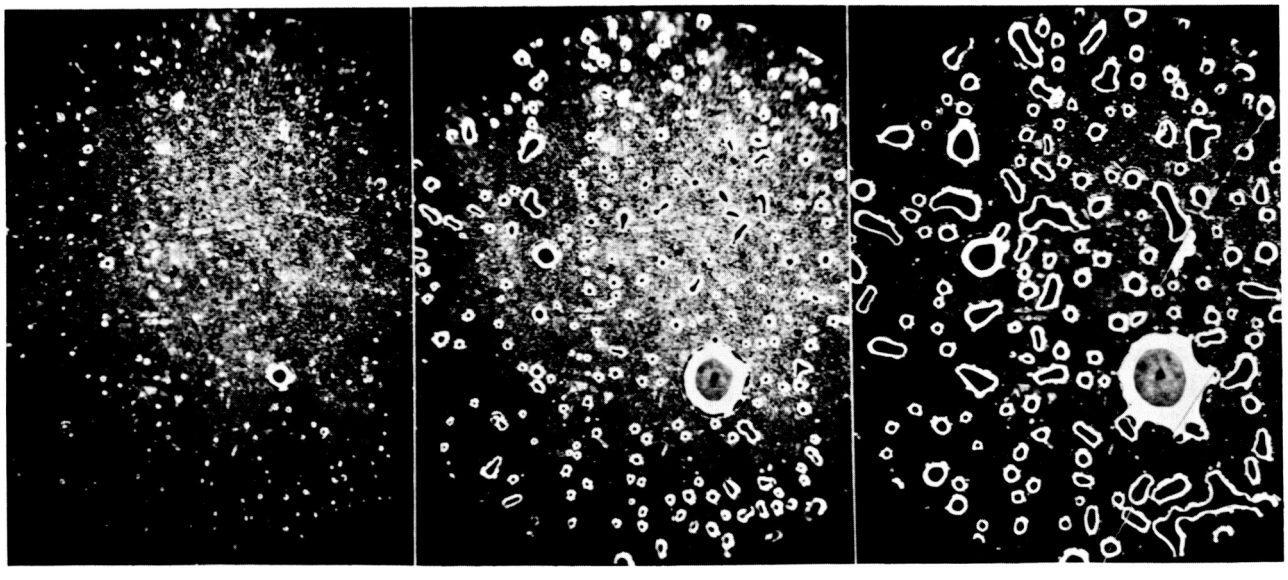

Fig. A. Photomicrographs of the same *E. coli* colonies after 4, 6, and 7 hr incubation (*left to right*) on a black membrane filter with reflected dark-field illumination. Field area 4 x 10^{-2} cm^2 ca. 50 x linear magnification.

Jannasch — How big were these round, colony-like structures on the filter?

Goetz — The largest colonies were about 0.03 cm = 300 μ in diameter. I'd like to mention also that at that time our group developed the instrumentation for applying this principal method by microphotometric recording at 20 to 30 minute intervals against magnetic memories to the point that a distinct discrimination between growing and neutral particles was possible after the first 40 to 60 minutes of incubation.

Jannasch — Did you transfer them to other media?

Goetz — No transfers were made. In our case he wanted only to see which of the particles would grow; we wanted to get as early a growth signal as was possible.

Greene — One of the frustrations that one faces when he has a small, but valuable sample is the question of priority. Is he going to examine the sample microscopically first, or is he going to ascertain viable count? He will be criticized in either case. If he examines microscopically before he plates, someone will object that he contaminated the sample. If he plates first, someone will object that he missed a chance to look for organisms that wouldn't grow under his specific cultural conditions.

We must realize that sometimes the sample we have is the only one we are going to get. The count may be only one per milliliter, and we only have a few milliliters. We cannot really divide the sample and run multiple tests on it. What priority do we give to the available methods of examination? And further, what medium shall we use if we have the choice of only one. When the count is extremely low, we may try to concentrate the viable material by membrane filtration. So the whole "sample" is on one filter. We cannot have the luxury of plating in a variety of ways. And if we make the "wrong" choice — we have no chance to repeat.

I suggest that this is one of the serious frustrations for which we stand in great need of consultation and help. Until we find the universal medium, until we find the ideal method for enumeration and examination, we're not going to satisfy everybody. Well, let's just face the fact that with our present knowledge we are doing as well as we possibly can.

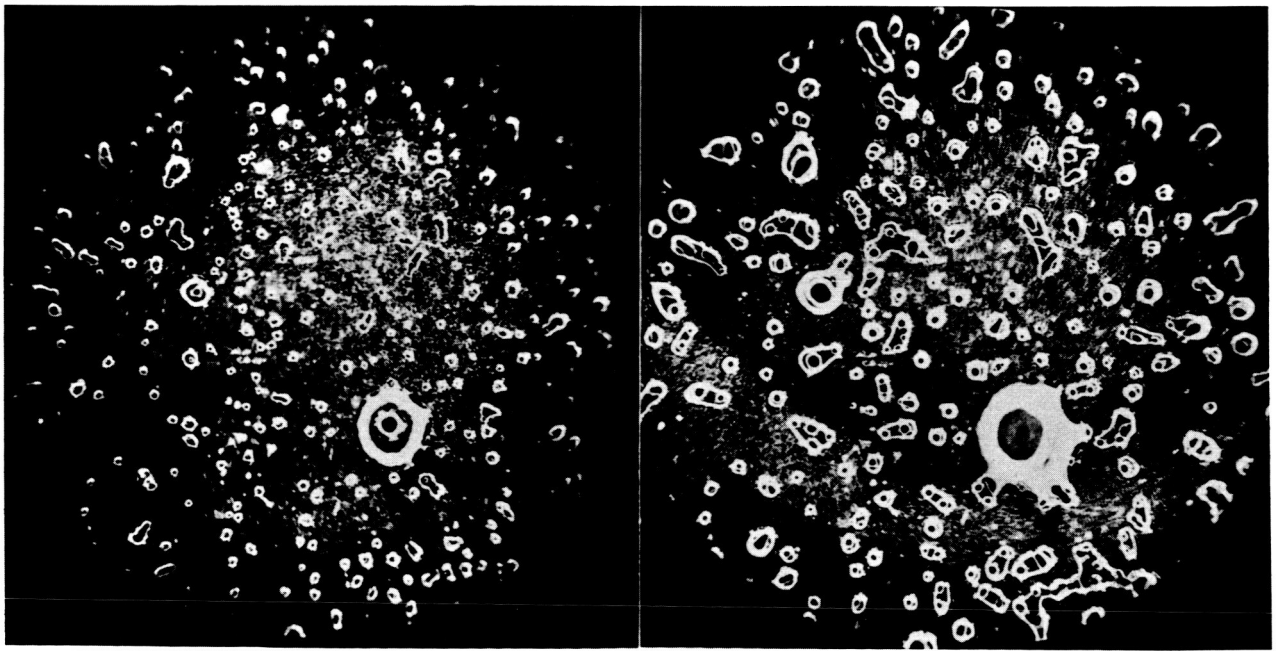

Fig. B. Superposition of photos in Fig. A: growth pattern (*left*) after 4 and 6 hr, same (*right*) after 6 and 7 hr.

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Atmospheric Particulate Matter of Plant Origin

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Abstract

23992

Studies of transport of pollen, spores, and other microscopic particulates of plant origin in atmosphere near earth's surface provide indications of potential transport of organic particulates to higher levels under various circumstances. For convenience, particulates of plant origin are grouped in following categories:

1. Small, light-weight propagules (spores, pollen grains, near-microscopic seeds)
2. Single- or few-celled plant bodies or fragments (bacteria, single-celled algae, fragments of fungal mycelia)
3. Deciduous tissue parts from plant surfaces (hairs, scales, resinous particles from macroscopic terrestrial plants)
4. Fragments of vegetable humus (material of plant origin degraded biologically or autolytically so that characters for determining origin are obscured)

Different kinds of particles in categories 1 and 2 remain viable in the lowest level of atmosphere for widely different time periods, from a few hours to more than a year. Both living and non-living particles are possible agglomeration nuclei or in some instances substrates for smaller parasitic or saprophytic organisms.

Plant origin particulates can be recovered from atmosphere near ground by static samplers, dynamic samplers, and devices for entrapping rain washout. Results of static sampling to obtain pollen spectra of modern vegetation are discussed.

Phytogeographical studies of seed and spore dispersal provide indications of transport distances. Geographic distributions of source plants for given kinds of particulates and distributions of different vegetation types determine patterns of sources from which air movements transport plant particulates. Source patterns are complex in detail because of habitat differences, and these patterns vary with seasons and with shorter term weather sequences.

From my experience in sampling airborne pollen and spores and other parts of plants in connection with studies of microfossils in sediments there occur to me certain comments and questions that seem pertinent to the mission of this conference, specifically, the following questions: 1) What is the potential yield of plant particulates to the atmosphere? 2) What particles are living? 3) Which particulates are propagules? 4) What conditions are limiting to particulate viability? 5) What conditions would permit the germination and establishment of viable particulates? 6) Finally, what nonliving materials might be significant as potential substrates for organisms, and under what conditions?

My remarks within the framework of these questions are limited to plant materials exclusive of bacteria and viruses. I plan to discuss briefly the varieties of vegetable matter particulates, remark about sampling that I have done, and finally, consider the geography of plant particulate yield.

Although there is considerable literature about the occurrences of organic particulate matter in the atmosphere, only bacteria, fungal spores, and spores and pollen of higher plants have been quantitatively sampled and systematically identified. Sampling has been confined to relatively few localities and short periods of time, with several notable exceptions; nearly all sampling has been in the lowest levels of the atmosphere. Obviously much remains to be done to diversify the sampling stations geographically and altitudinally, to improve sampling techniques, and to identify more of the entities recovered, if we are to obtain records approaching a census of airborne particles adequate for needs such as those that led to convening this conference.

In this paper the kinds of particulate matter of plant origin that can be potentially contributed to the atmosphere are grouped in an expedient classification, described in general terms, and evaluated with respect to occurrence and viability above the lowest levels of the atmosphere. Some recent experiences with sampling for pollen and spores in the atmosphere near the ground are discussed because selected results may provide guidance for upper atmospheric investigations. Finally,

the sources of plant particulate matter are surveyed from the phytogeographical and ecological viewpoints in an attempt to appraise the geography of vegetation contributing to the atmospheric load and potentialities for contamination of objects passing through the earth's atmosphere.

Categories of Particulates of Plant Origin

A. SMALL, LIGHT-WEIGHT PROPAGULES (SPORES, POLLEN GRAINS, AND DUST-SIZE SEEDS AND FRUITS). These particles are produced by plants for dispersal and propagation. Pollen grains are structures enclosing male gametophytes of a seed plant. Each grain is capable only of "fertilizing" an ovulate element of a flower of the same species. The several-celled gametophyte within the pollen grain remains alive for a few hours or a few days, succumbing more quickly under humid conditions. Spores are produced by the cryptogamic, or non-seed plants, including fungi, algae, mosses, and ferns. In the usage employed here, a spore is a propagule. The spores of most fungi are from 3 to 15 μ in maximum dimensions, although some are in aggregates that are considerably larger, with wide varieties of shapes and ornamentations. Moss (bryophyte) spores are for the most part between 6 and 30 μ, with or without a trilete scar, and with or without ornamentation of the surface. Fern spores are generally still larger, from 20 to 60 μ, marked by either a single straight scar (sulcus) or a trilete scar, and enclosed in a more or less obvious perispore layer that is ornamented differently in different genera and species. Many fungal spores are capable of retaining their viability for periods of some years under relatively extreme conditions of cold and dryness. Moss spores and fern spores remain viable for shorter periods as a general rule, and their tolerances of temperature and moisture conditions are probably narrower. Airborne spores of algae are mostly resistant cysts or "resting spores" in the size range of fungal spores. The term of their viability in absence of water and of other conditions for germination seems to be little known.

Seeds are propagules of flowering plants. A seed consists of an embryo resulting from the union of a male gamete (carried by a pollen grain) and an egg cell in a pistil-bearing flower, some stored food, and several protective integuments. A fruit consists of one or more seeds enclosed in more or less modified flower parts. Dust-size fruits are uncommon; small fruits equipped with hairs or other appendages that increase the surface area are easily carried by moving air. For convenience, the term seed will be used to cover both fruits and seeds. Seeds of most plants require special conditions of moisture, temperature, and in some cases light, for germination; the resulting seedlings have narrow tolerances of habitat conditions. Consequently the danger of extraterrestrial contamination by seeds is small. On the other hand, seeds may carry fungal mycelia, bacteria, or viruses that could be of concern.

B. SINGLE- OR FEW-CELLED PLANT BODIES OR FRAGMENTS (BACTERIA, ALGAE, FUNGAL MYCELIA, MOSS PROTONEMATA, AND LICHEN FRAGMENTS). I shall not consider bacteria here, but I cannot resist the comment that chemosynthetic bacteria deserve more investigation than they have been receiving. The iron or sulfur bacteria might well be capable of living in certain extraterrestrial habitats if water were available. Attempts to identify these organisms in air samples would probably encounter serious problems.

Single-celled algae in a resistant "resting" phase, fragments of filamentous forms, and fragments of more massive colonial algae are carried in a dry state by wind to different sites where they resume active growth when moisture, temperature, and light conditions are suitable. The protonemata that develop from germinating moss spores superficially resemble filamentous algae, and could conceivably be transported in a similar manner. The protonemata of some species, at least, might be expected to have no more exacting requirements for growth than the widely distributed terrestrial algae. Mycelia, the microscopic threads that make up the vegetative body of a fungus, are frequently found in air samples, but cannot be identified even to family or genus unless they can be cultured to the point of forming characteristic fruiting bodies. The viability of different mycelia while outside growth-permitting environments is poorly known, but it is likely that they are less resistant to environment stresses than are spores. Under certain conditions, undoubtedly, mycelial fragments are capable of propagation. Lichens, which are symbiotic aggregations of a (usually ascomycete) fungus and an alga (usually green), become brittle when dry so that fragments are readily broken from the plant bodies. Portions of the lichen thallus can survive drying for years and can re-form the characteristic whole plant body when growth is resumed.

C. DECIDUOUS TISSUES AND SECRETIONS FROM PLANT SURFACES (HAIRS, SCALES, RESINOUS AND WAXY PARTICLES, AND LEAF FRAGMENTS FROM MACROSCOPIC TERRESTRIAL PLANTS). The significance of these particles is that, at sizes of more than 1 or 2 μ, they could conceivably form nuclei for the agglomeration of bacteria or spores of smaller size, and as organic matter they are potential substrates for saprophytic bacteria and fungi. Hairs, scales, and the general cuticle that covers the aerial portion of a vascular land plant are composed largely of a waxlike material called cutin, which in turn is usually covered with one or more waxes. These substances are mixtures of free hydroxy fatty acids with their wax and soap condensation products. They are more resistant to decomposition by fungi and bacteria than are proteins and carbohydrates, but are attacked in time under aerobic conditions in the presence of water. Some plants secrete minute globules of waxes and resins on their surfaces, and many release hydrocarbon vapors. Went (29) has developed evidence that summer haze consists of peroxides and ozonides of these hydrocarbons formed under influence of sunlight in the presence

of nitrogen oxides. Initially the particles are of sub-micron size, and they agglomerate to form increasingly larger particles. Finally they become stable, bitumin-like particles several microns in diameter and settle out of the atmosphere. Volatile hydrocarbons from vegetation might be one source of haze particles of the kind Dr. Goetz describes in his paper for this Atmospheric Biology Conference.

Woody plants produce corky bark on the larger portions of the main axes. Walls of cork cells contain suberin, a substance that is somewhat like cutin in its composition and properties, and is similarly resistant to degradation by bacteria and fungi. Cork cells, singly and in small fragments of tissue, are found in samples of atmospheric particles near the ground level almost as commonly as plant hairs and other bits of cuticularized material. Bending of stems in wind probably releases bark and other dead surface tissue fragments to the air.

It is possible that some of the particles in this category, especially in size ranges small enough to have a large proportion of reactive surface, undergo changes in chemical structure to form particles resembling those described in the following category.

D. FRAGMENTS OF VEGETABLE HUMUS (PLANT ORGANIC MATTER THAT HAS BEEN DEGRADED TO THE POINT THAT ITS ORIGINAL MORPHOLOGICAL FEATURES ARE NO LONGER DISTINCT). In samples of atmospheric particulates we often find a considerable proportion of material that can be described only as amorphous vegetable humus. It is orange-brown to amber colored depending upon the thickness of the particle and the amount of light transmitted. It is firm and can be attacked by only strong oxidizing or hydrolyzing agents such as nascent chlorine, a hypochlorite, concentrated sulfuric acid, or strong mineral hydroxide. It will burn or char; in many of its properties it resembles the matrix of lignite or low grade bituminous coal. This highly degraded vegetable humus could conceivably be transported by wind or water, deposited, re-transported, and re-deposited several times before falling into the situation in which sunlight and weathering or biological agents finally break it down.

Static Sampling of Sediments from Atmosphere

Palynologists who investigate fossil assemblages of pollen and spores from the relatively recent geologic past inquire empirically into modern sedimentation of those particles from the atmosphere in order to make better interpretations of vegetation and environmental conditions on the basis of microfossil assemblages. Reconstructing past conditions by applying knowledge of present organism-environment relationships is the basic principle of paleoecology. Some of the techniques used for sampling modern pollen and spore "rain" provide information of significance to the problems of atmospheric particulates that concern us in this conference. Dr. Gregory has provided a complete survey of both static and dynamic sampling techniques in his book (15), but I can add here some comments based on experiences in attempting to relate atmospheric loads to existing vegetation and to recent sediments.

Common approaches to problems of contemporary sedimentation from the atmosphere for paleoecological purposes include sampling the most recent sediments of lakes (11,21,5) or stock tanks (16) and sampling organic debris lodged in the mesh of moss or lichen mats (9,22,2,17). Moss (bryophyte) polsters in bogs and in forests on moist sites provide humid acidic lodgment places for pollen and spores, which decay slowly or not at all under those conditions, so that assemblages, representing several years to several decades of sedimentation, accumulate.

I used moss polsters in a study of variation in pollen rain in different plant communities on one bog about ten miles southeast of the Straits of Mackinac in Michigan (2). Variation of pollen spectra was considerable from one community to another, but the variability was attributable primarily to yields from shrub and herb layer species rather than to tree species. Short distance transport was evident, however, even for low-stature and entomophilous (insect pollinated) species so that the pollen and spore sediment represented the enclosing plant community in only a general way. A relatively constant background in the spectra consisted of pine, oak, beech, hemlock, and maple tree pollen along with pollen of the grass family and the composite family; these were considered part of a *regional* pollen rain. Genera such as spruce, birch, and balsam fir were present in this regional component, but they showed fluctuations attributable to proximity of stands on or bordering the bog. Sedge family, heath family, and arbor vitae pollen were reliable indicators of stands close to the site of deposition. Larch tree and cat-tail pollen, and marsh fern and sphagnum spores, were less reliable indicators of presence of those plants in the immediate vicinity. As in similar investigations, this study demonstrated a complex spatial pattern of atmospheric sedimentation. Different times of pollen or spore production by different species coupled with variations in surface winds are primarily responsible for the complexities of these patterns.

Natural collecting surfaces such as moss polsters are not suitable for short term sampling, they are far from uniform in their collecting and preserving properties, and they often are not growing where sampling is required.

In an effort to find a device that would simulate the moss polster as a collecting surface, Mrs. Darlene E. Southworth and I made trials with petri dishes of glass beads coated with different adhesive substances (Fig. 1). The increased and irregular surface was more efficient than were

flat glass slides for at least the smaller sizes of pollen grains, but the dishes required protection from rain. A rain shield would prevent collection of material washed out of the air by rain (cf. 13) and would interfere with direct fall of particles in dry weather. Dr. Estella B. Leopold of the U. S. Geological Survey is testing the standard Weather Bureau rain gauge as a collector of pollen sedimenting from the atmosphere. The rain gauge would be expected to be suitable for collecting rain washout pollen, but its efficiency for collecting from dry air during windy periods is still questionable.

An adhesive coated mesh sphere seemed to me potentially useful, because moving air and falling rain could enter and would lose velocity, increasing the probability that both heavier and lighter pollen grains would impinge or sediment upon sticky surfaces of the mesh. It would also simulate inexpensively the fine permeable mesh of vegetational surfaces such as that of moss colonies. The Tuffy plastic mesh scouring pad manufactured by General Foods appeared on the market about this time, so we ran several tests in the summer of 1961, with results that indicated collecting efficiency greater than that of petri dishes of adhesive coated glass beads and retention of pollen during, if not from, falling rain (Fig. 1). The mesh spheres were coated by dipping in melted glycerine jelly or silicone oil. They were then drained before being individually packaged in glassine bags and left until exposure at the collection site. After 7 days exposure in warm sunny weather or freezing weather, glycerine jelly had lost some of its adhesive property, but particles once caught seemed to remain attached. Silicone oil gradually drained off until, after 1 week, only a thin film remained; yet the collecting efficiency compared favorably with that of glycerine jelly. At the end of the exposure period, the spheres were again sealed in clean glassine bags and returned to the laboratory, where the adhesive and trapped particles were washed free of the mesh. The glycerine jelly was washed off in boiling water; the silicone oil, in boiling benzene. Following this extraction, the suspensions were centrifuged, washed, and then prepared as desired for identification and counting under the microscope. For inspection of the total collection with minimal treatment, a 2% aqueous solution of trisodium phosphate deflocculated the sediment and restored pollen, spores, and bits of tissues to a turgid, expanded state (1).

We believe that glycerine jelly is superior to silicone oil as an adhesive, but that some glycerine jelly is washed off by long and hard rains. To retain dislodged pollen and a greater proportion of the rain washout fraction we propose to mount a cone of filter paper on the underside of the mesh sphere. In summary, coated mesh spheres offer some advantages over previously employed static samplers and are potentially useful devices for reconnaissance surveys.

Mrs. Southworth and I restudied the fossil pollen in a long core from Third Sister Lake on the southwest side of Ann Arbor to amplify the results obtained by Potzger and Wilson (23) on the tree pollen alone. Sediments of the uppermost 3 ft are believed to contain the records since clearing for agriculture about the middle of the last century, but the pollen record is difficult to interpret. Therefore we undertook collection of modern pollen rain from April to November in order to have comparative information from the present landscape.[1] By using mesh spheres and rain gauges in parallel as samplers we accumulated information on the modern pollen rain as well as information that will allow evaluation of the relative efficiencies of these two devices under various conditions of rain, freezing, and wind. Third Sister Lake is within the University of Michigan Saginaw Forest, an experimental forest of 66 acres upon which detailed records of plantings and other management practices have been kept for 60 years.

We established the following program of sampling, with two stations, one at each end of the lake (approx. 350 meters apart, Fig. 2) as follows:

A. Weekly records of plant species in anthesis throughout the forest property.

B. Weekly samples from:

1. Station at east end of lake
   a. Rain gauge
   b. Mesh sphere coated with glycerine jelly
   c. Mesh sphere coated with silicone oil
2. Station at west end of lake
   a. Rain gauge
   b. Mesh sphere coated with glycerine jelly
   c. Mesh sphere coated with silicone oil

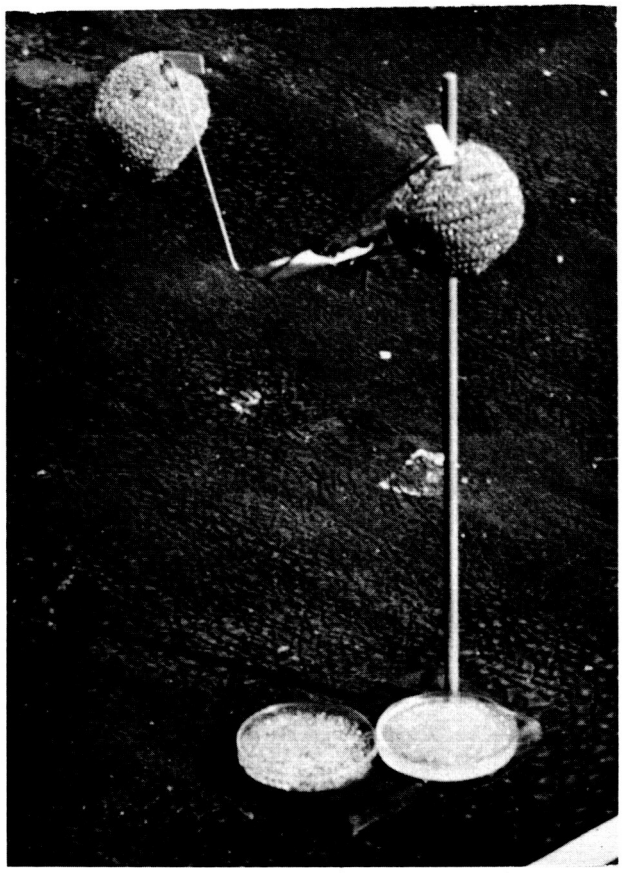
C. Monthly samples of lake water from surface 2 cm and from 100 cm depth (15 July, 15 August, 15 September, 15 October):

1. East of center
2. Center
3. West of center

D. Monthly samples of lake water from surface 2 cm and samples of bottom flocculent sediment (at c. 30 cm water depth) (15 July, 15 August, 15 September, 15 October):

1. 15 Meters offshore from east end
2. 15 Meters offshore from west end

[1]This study was assisted by a grant from the Horace H. Rackham School of Graduate Studies of the University of Michigan.



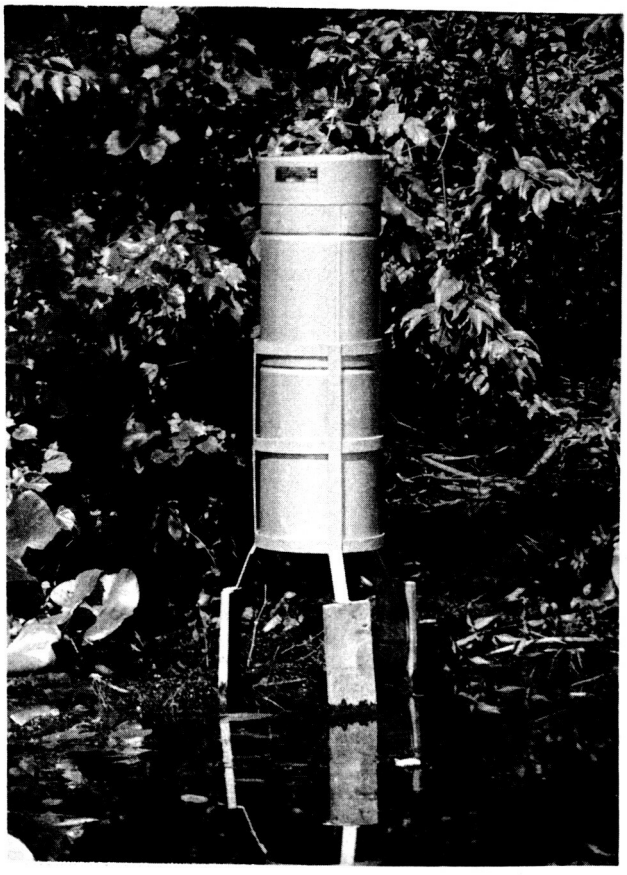


Fig. 1 *(left)*. Trial devices for static sampling of airborne pollen and spores. Petri dishes with glass beads (two dishes shown here) contained glycerine, glycerine jelly, silicone oil 1,000 centistokes viscosity, silicone oil 12,000 centistokes viscosity, silicone oil 12,000 centistokes viscosity, and Tanglefoot. Suspended above are two Tuffy plastic mesh scouring pads: *left*, coated with glycerine jelly, *right*, coated with 12,000 centistokes silicone oil. August 1961.

Fig. 2 *(right)*. West station for static sampling airborne pollen and spores at Third Sister Lake. Two Tuffy mesh sphere samplers can be seen above and to the left of standard rain gauge. August 1962.

Of the 220 samples collected for pollen analysis, nearly half have been prepared and analyzed. In Table I are shown mesh-sphere and rain-gauge sample results for the week of May 30 to June 5, 1963. Only the sums are shown for non-arboreal pollen counts. The variance in numbers of pollen in each category is more than we had expected, but hopefully it will be instructive with regard to the relative efficiencies of the three kinds of samplers, relative concentrations and dispersal differences of various pollen entities, and influences of weather and site conditions on sedimentation from the atmosphere. Pollen statistics from the suspended sediment and bottom sediment collections on the lake are expected to aid in elucidating the sedimentation process in the water. Publication of the complete results is probably 2 years away.

The point here is that in the lowest levels of the atmosphere there is an intricate and Protean mosaic of pollen and spore populations, at least during the flowering season. Other organic particulate matter of the kinds described above undoubtedly occur in similarly complex and changing patterns.

The *Eucalyptus* pollen (Table I) was added as 0.4 ml of a standardized suspension in glycerine to all of the samples, in order to provide a proportional volume measurement in the counting process (3). We had determined by means of hemacytometer counts that the stock suspension contained 272,000 *Eucalyptus* grains per milliliter ± 30%, so the 0.4 ml added 108,800 ± 30% to each sample. The variance in the *Eucalyptus* counts was high because of clumping of the small grains and poor pipetting methods. We subsequently improved the pipetting methods and changed to the larger pollen of *Cycas* which does not clump, so that we now have a stock suspension containing 127,500 grains per milliliter ± 5%. This additive technique has proved useful in determining through the microscope count what aliquot of the total sample has been inspected, and it also has provided a means for comparing the total pollen and spore populations of different samples.

Although our air sampling study at Third Sister Lake was designed to comprehend the part of the year when local plant species were in anthesis, Mrs. Southworth and I took some samples of

Table I.

Pollen collections at Third Sister Lake, Ann Arbor, Michigan *

	West station									East station								
	Si oil mesh sphere			glyc jelly mesh sphere			Rain gauge			Si oil mesh sphere			glyc jelly mesh sphere			Rain gauge		
	No.**	%AP	% P	No.	%AP	% P	No.	%AP	% P	No.	%AP	% P	No.	%AP	% P	No.	%AP	% P
Picea	0			3	2.1	1.1	3	2.9	0.5	0			1	0.5	0.2	0		
Pinus	9	28.1	5.6	12	8.3	4.4	14	13.5	2.5	61	31.0	12.1	21	10.2	4.2	13	12.4	2.5
Salix	1	3.1	0.6	4	2.8	1.5	1	1.0	0.2	4	2.0	0.8	3	1.5	0.6	18	17.1	3.5
Quercus	10	31.3	6.2	67	46.2	24.5	48	46.2	8.7	94	47.7	18.6	129	62.9	25.9	41	39.0	7.9
Fagus	0			2	1.4	0.7	2	1.9	0.4	2	1.0	0.4	1	0.5	0.2	6	5.7	1.2
Carya	2	6.3	1.2	2	1.4	0.7	3	2.9	0.5	16	8.1	3.2	6	2.9	1.2	11	10.5	2.1
Juglans	7	21.9	4.3	2	1.4	0.7	8	7.7	1.4	15	7.6	3.0	7	3.4	1.4	8	7.6	1.5
Acer																		
saccharum	0			11	7.6	4.0	7	6.7	1.3	0			7	3.4	1.4	0		
ginnala	2	6.3	1.2	0			12	11.5	2.2	0			6	2.9	1.2	0		
rubrum	1	3.1	0.6	1	0.7	0.4	1	1.0	0.2	0			2	1.2	0.4	0		
negundo	0			0			0			0			1	0.5	0.2	0		
Fraxinus	0			7	4.8	2.6	0			0			5	2.4	1.0	0		
Betula	0			14	9.7	5.1	5	4.8	0.9	5	2.5	1.0	9	4.4	1.8	7	6.7	1.4
Carp./Ostrya	0			0			0			0			0			0		
Populus	0			4	2.8	1.5	0			0			1	0.5	0.2	0		
Ulmus	0			16	11.0	5.9	0			0			6	2.9	1.2	1	1.0	0.2
ΣAP	32	100.1	19.8	145	100.2	53.1	104	100.1	18.8	197	99.9	39.1	205	99.9	41.1	105	100.0	20.3
ΣNAP	130	406.3	80.2	128	88.4	46.9	449	431.7	81.4	308	156.2	61.0	293	142.9	58.7	412	39.4	79.8
ΣP	162	506.3	100.0	273	188.4	100.0	553	531.7	100.2	505	256.1	100.1	498	242.9	99.8	517	492.4	100.0
Eucalyptus	500	1562.5	308.6	500	344.8	183.2	500	480.1	90.4	500	253.8	99.0	500	243.9	100.4	500	476.2	96.7

* May 30-June 5, 1962. Non-arboreal pollen entities represented here only by the sum (ΣNAP) for each sample. Extracted from unpublished data of Benninghoff and Southworth.

** No. is number of grains per 500 added *Eucalyptus* grains. *Eucalyptus* count is not included in total pollen count (ΣP). %AP is percentage of total arboreal pollen count. % P is percentage of total pollen count.

snow 5 February 1962, the day after a winter storm, out of curiosity about the possibility of pollen being deposited with the snow. Two samples of fresh snow were taken on the ice of Third Sister Lake and one sample in a woods 3 miles to the northeast. The richer sample from the lake contained approximately 1,000 grains per liter of water equivalent, with elm, hickory, pine, oak, cat-tail, and grass pollen predominating. The woods sample contained only about a third the density of grains and a considerably poorer representation of non-arboreal pollen grains. Pollen in snow from the lake ice surface must have been dislodged from tree, shrub, and dead herb surfaces and blown onto the lake along with snow particles; perhaps the snow exerted some scouring effect. We have thought of the possibility that some of the pollen that is filtered out by forest stands during the period of anthesis (cf. 12) have actually lodged on leaves, twigs, and bark, only to be carried to the forest floor by rain-wash, leaf fall, and snow scour at later times. We need definitive surveys to confirm these ideas, but the suggestions are evident that although vegetation, especially shrub and forest stands, act as barriers to horizontal transport and as sedimentation traps, they may also act as sources from which once sedimented pollen and spores can be re-launched into the atmosphere.

These observations on pollen and spores in temperate latitude snow contrast markedly with observations of polar region snow and glacier ice. For example, L. H. Nobles, H. C. Robbins, and I carefully excavated approximately 1/4 m^3 of old glacier ice 4 miles out on the Greenland Ice Cap northeast of Thule in 1953 in a search for pollen and spores. By melting, decanting, and finally centrifuging we recovered a minute quantity of sediment — mostly fibers from our clothing, some appendages of tiny arthropods, a few fragments of mycelial and algal filaments, and two spores believed to be from fungi.[1]

Phytogeographical and Ecological Considerations

Gregory (15, pp. 181-193) treats the general subject of long distance dispersal of diaspores, with particular emphasis on phytopathogenic fungi, but including evidence such as the interesting record of revegetation of the island of Krakatoa following the sterilizing eruption of 1883. We need more sampling of the atmosphere and more critical determinations of collections, of course, but it may be of some use to consider a few aspects of the geography of plant groups that produce airborne particles

[1] These and other observations were made in Greenland by the author while he was assigned by the U. S. Geological Survey as Botanist to the U. S. Army Project Ice Cap in 1953.

of potential concern in the space vehicle microbiology problem. The point to be made here is that knowledge of the occurrence and relative density of the various plants that yield particles to the atmosphere will permit quantitative prediction of atmospheric loads for given areas and interpretation of travel distances for identifiable materials caught in air samples (cf. 28, 24).

Seeds of vascular plants I think are generally of less concern as possible contaminants, because of the relatively exacting ecological requirements for germination and growth. But as Ridley (25, Chap. I, Dispersal by Wind) has demonstrated so vividly, dust seeds and plumed and winged seeds are transported both horizontally and vertically for distances approaching those of the larger spores and pollen grains, and all of these organic materials can serve as nucleus particles for transport of diaspores of potential concern, and all are potential substrates for growth of saprophytic organisms. Seeds of orchids are outstanding examples of the dust size group, yet most orchids have highly restricted ranges both geographically and ecologically. The seeds germinate and the protocorm succeeds in growing only under very special conditions (cf. 27), so they are not likely to be troublesome contaminants. *Epilobium angustifolium*, the fireweed of boreal regions, has a small plumed seed that can be borne by the lightest breeze. The plant appears in crowded colonies over thousands of acres of newly burned conifer forest land within one or two years, and the species is circumboreal, extending from the southern part of the Arctic down to the middle latitudes. Nevertheless the plant occurs only in a selected group of habitats throughout this great range. I have watched concentrations of airborne fireweed seeds settle out in forest, shrub, and grass stands just as we know microscopic particles to do, and I have seen the air completely scavenged of the seeds by a rain shower of only a few minutes duration. These plumed seeds that are so easily observed might be useful models for studies of air transport and sedimentation if we were to watch them carefully.

Plant geographers are by no means unaware of the power of atmospheric transport of diaspores, and call upon this device whenever other hypotheses fail to explain puzzling disjunctions of range. The greater proportion of fern species in the floras of isolated oceanic islands has been ascribed to the combined facts of the likelihood of atmospheric transport of the diaspores (20-80 μ, for the greater part of the species) and moist equable climates that favor germination, growth of the gametophyte, sexual reproduction, and growth of the sporophyte (30).

Aside from interest here in pollen as organic substrate material and as nuclei for smaller diaspores, records of pollen in air samples are useful because most kinds can be identified to family, many to genus, and some even to species. Thus they can be traced back to source plant colonies and to specific time periods of anthesis.

Seeds, pollen grains, spores, and even larger plant fragments can be transported in small or large numbers over very great distances by uncommonly strong winds and by being carried to great heights by updrafts in violent storms. In early June, 1951, I observed the effects of an unusual shower of Sitka spruce pollen along the Pacific Coast from Ketchikan to Juneau, Alaska. Flying north over the Inside Passage route at about 8,000 ft in clear sunny weather, I could see great yellow patches and swirl patterns at the mouth of every stream. At Juneau I inspected the waters of bays and estuaries, and found that Sitka spruce pollen occurred in continuous patches 1 to 3 mm in thickness over areas as large as a quarter of a square mile. I was told that rain and wind dispersed the pollen masses on the water several days later.

A different aspect of the geography of vascular plants that could have significance is that of regional differences in the incidence of certain morphologic features, which have rather general but nonetheless valid correlations with vegetation formations and regional climates. For example, plants bearing copious hairs, epidermal scales, and waxy atoms on their surface are more common in arid regions (deserts, grasslands, or savannas) than elsewhere. Forest vegetation yields few dust seeds to the atmosphere, trees produce generally larger and heavier fruits and protect the small herb seeds from being carried into the atmosphere. Broad-leaved forests yield fragments of leaves, and, especially while they are leafless, bits of cuticle, bark, and (in spring) hairs from buds and young leaves. Broad-leaved and needle-leaved evergreen forests yield similar particulates in lesser quantities, but conifer forests are often infected with needle-rusts that produce large numbers of small diaspores. Therefore, it might be possible to assess different land areas of the world with respect to characteristic potential production of particulate matter, including the several categories of particles enumerated above. Where the landscape is intricately patterned, as in areas of high relief or in agricultural areas, the problem would be complicated, but there would also be greater numbers of characteristic particulates to draw upon.

Gregory (15) has presented a thorough survey of the geography and atmospheric transport of fungus spores, but with emphasis on the phytopathogenic forms. The geography of the fleshy fungi, the larger-fruited forms of the Ascomycetes and Basidiomycetes, deserve some attention also. Few works treat the general geography of the fungi (6), but I have permission to convey the following information from lectures Prof. Alexander H. Smith presents each year to my phytogeography class.

The first problem in the geography of the fleshy fungi is that we cannot know of the presence of a given species until it produces a fruiting body, such as the mushroom form or puffball form, in or above the surface of the wood or humus in which the minute, threaded white web of mycelia is growing. Some mycelia of actually common species of woodland fungi may go for long periods without fruiting. With the advent of the proper moisture and temperature conditions, fruiting may be abundant for several days to several weeks. Spores are produced in fantastically large numbers by many species. In preparing suspensions of spores for adding to fossil pollen preparations for proportional volume control, we have obtained more than 5 billion

spores from a single fruiting body of *Pisolithus tinctorius* (somewhat like a columnar puffball). The spores are approximately 5 x 4 μ and slightly flattened.

All fungi are more or less restricted by food specialization, that is, restricted to growth on specific substrates. The fleshy fungi follow mostly distinctive seed plant communities; some are parasitic on single species of phanerogamic plants. Like the lower fungi these can survive under widely different climates, as desiccation does not kill the mycelium, at least for one to several years. In general the fruiting bodies are less succulent and more woody in desert areas where the hymenium needs protection from sudden drought, but in the moist tropics the fruiting bodies are smaller, and produce fewer spores. Fruiting is cyclic in the temperate regions; some species characteristically fruit in spring, or summer, or autumn. In the dry tropics fruiting is related to the availability of moisture, and in the moist tropics there are no evident seasonal cycles.

According to A. H. Smith, the floras of fleshy fungi may be grouped according to the following world distribution patterns: world-wide or nearly so, north temperate (associated especially with conifers), tropic, south temperate-antarctic, and warm desert. Consequently the atmospheric load of spores from the fleshy fungi will vary between those world regions, and within them according to different vegetation cover, local patterns of habitats, the seasons, and weather sequences.

The microscopic and near-microscopic algae deserve mention here because of their widespread occurrence, their simple forms as autotrophic plants, and the potentialities for their distribution through the medium of the atmosphere (8, 19). Works devoted to world distributions of the algae other than the marine macro-algae are yet to be written. For the following statements on distribution and ecology of algae, except where credited to other sources, I am indebted to Prof. Wm. Randolph Taylor who lectures to my phytogeography class on these topics.

By and large endemism is uncommon among the more minute algae. Both genera and species of fresh-water and terrestrial algae have wider distributions than the comparable groups in the marine algae, which tend to be restricted to major ocean current systems. The appearance of narrow endemism in records of algae has usually been found to be an artifact of incomplete collecting. There seems to be more endemism around the Antarctic continent. Australia, New Zealand, and Tasmania seem to have a relatively distinctive flora. Isolated oceanic islands do not have rich floras. Planktonic algae are more widely uniform in distribution over the world than are attached bottom-dwelling forms. Propagules of marine algae generally do not resist drying, so transport in the atmosphere is unlikely to play a role in dispersal of those algae.

The vegetative parts as well as the resistant spores of many of the fresh-water algae have remarkable survival powers. *Gleocapsa* can survive in the dry state for more than a year without spores having been formed. Temperature conditions are limiting to the growth of many fresh-water algae, as they ordinarily grow in waters with relatively stable temperature regimes. In the spore condition or the dry vegetative condition many can probably survive low temperatures.

The blue-green algae are the most uniform in distribution over the world, but they show the greatest diversity in the tropics. Admittedly this group, and the other algae as well, are still poorly known in the Southern Hemisphere. Lakes and streams of temperate regions show the most generalized floras of all. For example, there is strong similarity between the fresh-water algal floras of temperate Asia and temperate North America. The algae that live in snow and ice, such as the commonly found "red snow" organism, *Chlamydomonas nivalis*, are found quite generally distributed in arctic and nival alpine habitats of the Northern Hemisphere (Fig. 3). There are relatively small differences between these boreal snow and ice floras and their counterparts in the Southern Hemisphere (14, pp. 167-172; 18). Undoubtedly these algae, as fragments or spores, are widely distributed in the atmosphere.

On marginal areas of the Ice-Cap in northwest Greenland, Nobels and I investigated pits containing living colonies of filamentous blue-green algae (*Calothrix parietina & Anacystis limnetica*, identified by Dr. Francis Drouet) (4). Each winter, and from time to time during the summer, these colonies are frozen solidly into the ice at depths of 20 to 65 cm, but with a day or two of insolation, the dark reddish-brown colonies heat up and thaw toward the surface columns or lenses of water so that the plants can resume growth (Fig. 4). After several days of solar warming the colonies lie at the bottoms of open, water-filled pits from 1 to 300 cm in diameter, depending upon the size, and presumably the age, of the colony. We found fragments of algal filaments in cryoconite pits (formed by aggregations of dust mixtures) and in samples of apparently clean ice; so we assume that the algal filaments are distributed by wind as well as melt-water on the glacier surface. Algae capable of living under conditions such as this would be good candidates for survival in some extraterrestrial habitats, and possibly candidates also for growth if water and sunlight were available.

Soils support considerable algal floras, especially of the blue-greens but including some green algae and diatoms (19,20,7). F. E. Fritsch and others in England made extensive studies of soil algae a half century and more ago (10). The soil algae were found to possess archaic modes of reproduction and are therefore difficult to identify. They are also difficult to grow in pure culture. Damp soils of cool-moist and alpine regions have abundant soil algae. Snow flush areas in arctic and alpine tundras commonly have large mats of *Nostoc* and other genera growing on the surface among the higher plants. Soil algae are especially abundant in the tropics, filling widely different roles in the ecosystems; some are important in the reduction of organic matter. The uppermost layers of many desert soils have been found to contain algal floras that are highly significant with respect to stabilization of the surface and fixation of atmospheric nitrogen (26). Organic crusts on intermittently watered soil and rock surfaces in arctic regions such as northern Greenland indicate similar activity by algae, but I have been unable to secure samples that contained

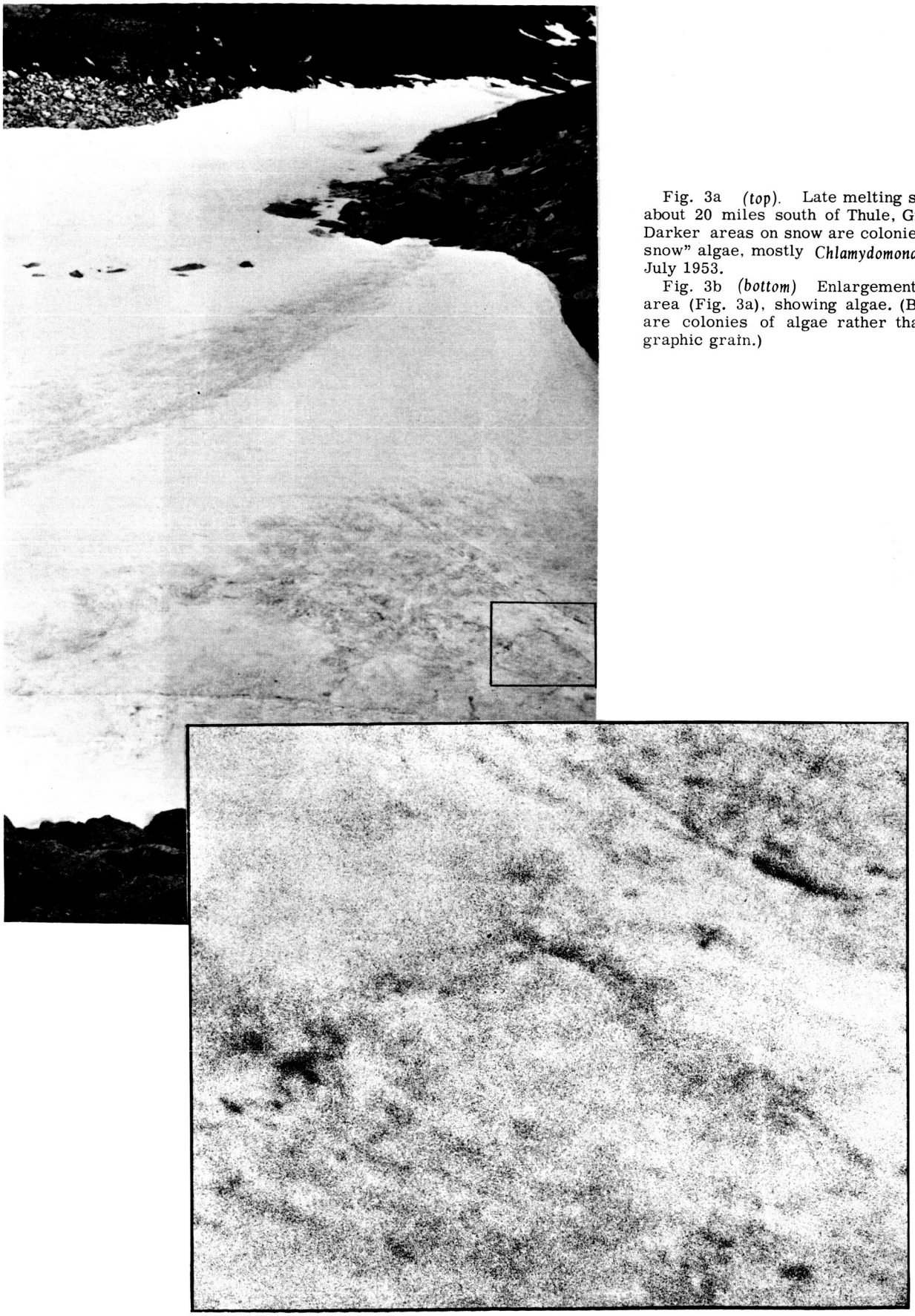


Fig. 3a *(top)*. Late melting snow bank about 20 miles south of Thule, Greenland. Darker areas on snow are colonies of "red snow" algae, mostly *Chlamydomonas nivalis*. July 1953.

Fig. 3b *(bottom)* Enlargement of boxed area (Fig. 3a), showing algae. (Black dots are colonies of algae rather than photographic grain.)

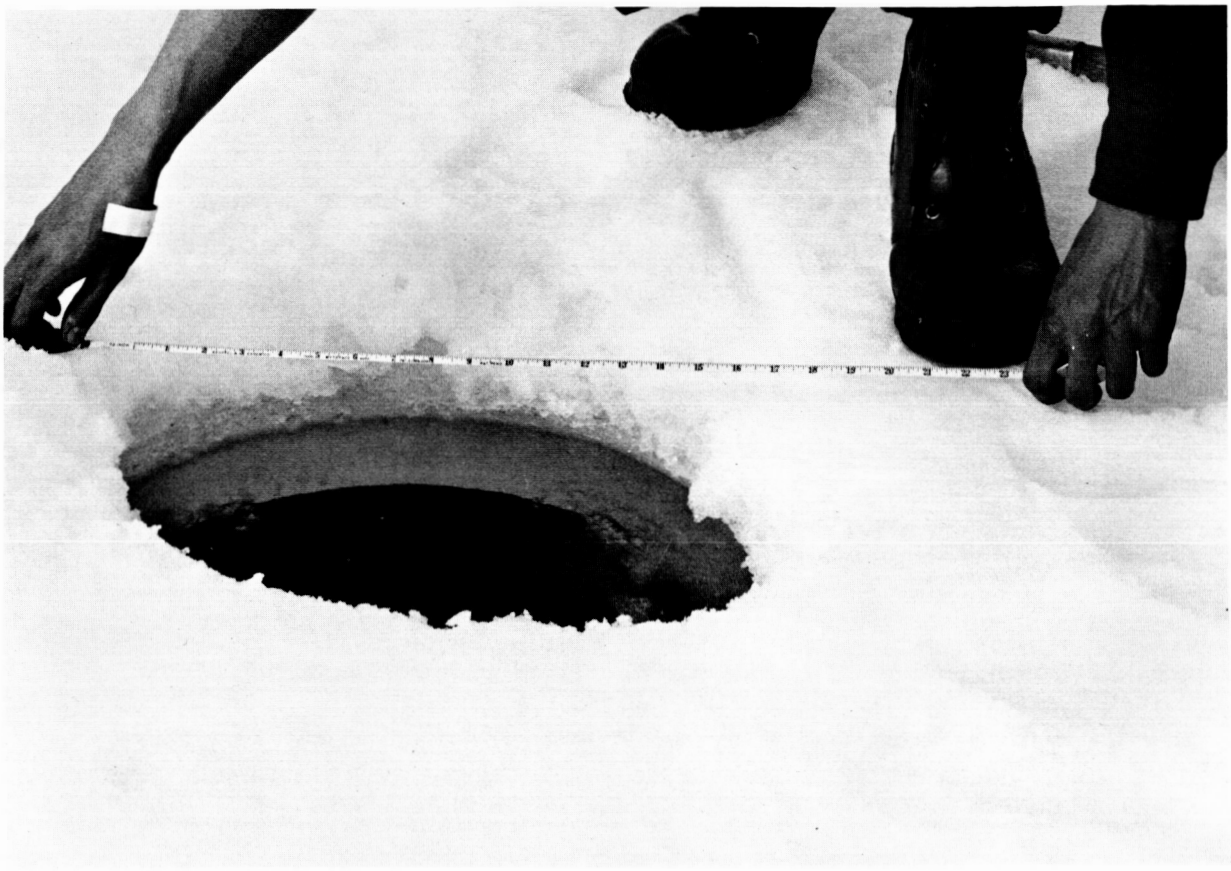


Fig. 4. Algal pit of common form on Greenland Ice Cap northeast of Thule. Community of algae can be seen as dark sediment at bottom of clearwater pool. August 1953.

material suitable for positive identifications even by specialists. The soil algae, like the snow and ice algae, are likewise good candidates for survival in extraterrestrial habitats. B. Muriel Bristol (7) described experiments with various soil samples which upon being moistened after 22 to 69 years of dry storage each produced a succession of bacteria, unicellular green algae (in some instances along with a few moss protonemata), and lastly several species of blue-green algae that quickly become dominant.

Lichens certainly include some of the most hardy plants with respect to resistance to environmental extremes; crustose and foliose forms are found at the very limits of plant growth toward the poles and on the highest alpine surfaces (Fig. 5). Although lichens do produce spores from their fungal components, with fragments of algal components occasionally being shed with the spores, the more common means of reproduction is by somewhat specialized fragments called soredia or by thallus fragments. One might expect soredia to be transported in considerable numbers in the atmosphere, but soredial bodies either are not caught in air samples or are not recognizable as such. Fragments of lichen thalli are sometimes caught, but may have been transported only short distances and near the ground (cf. 15, pp. 150-151). Nevertheless lichens are autotrophic, extremely hardy plants of relatively unfertile sites widespread on all continents; those on exposed plains and mountains are favorably situated for being launched into at least the lower atmosphere.

Conclusions

The object of this paper is to draw attention to three main points of importance in considerations of atmospheric biology in light of contamination of objects, including vehicles, that may transit the thickness of the atmosphere.

I. Particulate matter of plant origin is introduced into the atmosphere near the ground in enormous variety and in large quantity. Most of the entities are as yet unidentified, and the potentially viable entities are only now coming under study.

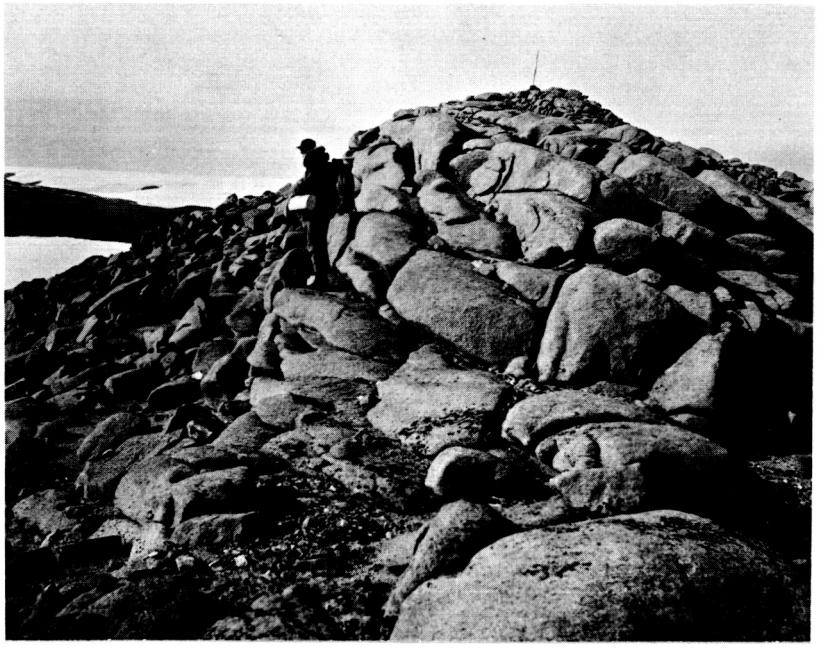


Fig. 5. Small nunatak (ice-free eminence) more than 2 miles within Greenland Ice Cap northeast of Thule. Plant life consisted of 5 species of lichens and 2 species of mosses. August 1953.

II. Identities and population densities of the different kinds of particles vary markedly over small areas near the ground and over regions of sub-continental size at higher (but still undetermined) altitudes in the troposphere.

III. The atmosphere near the solid earth surface carries different organic particle loads in different areas, varying with seasons and varying with shorter term weather sequences. It should be possible to map the kinds of particle yield to the atmosphere from different continental areas after a network of observing stations over the world has been in operation for several years.

Once the regional yields of plant particulates have been charted we can combine knowledge of plant distribution patterns with knowledge of vegetational phenology and meteorological phenomena to predict atmospheric loads for given regions. If laboratory culture work can be simultaneously expanded to provide knowledge of environmental conditions for germination and growth of the viable components, we shall then begin to take in hand the problems of controlling biological contaminaton of atmosphere-transiting vehicles.

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Discussion

MacLeod – I want to ask a question about climatological aspects of plant geography. Perhaps you were not leading to this directly, but it seems to me that in your pollen studies, you are relating species occasionally to a paleoclimate. Would you like to comment on that, perhaps with reference to other data that might support the specified paleoclimate?

Benninghoff – The main principle of paleoclimatology is interpretation of fossils and their enclosing deposits on the basis of uniformitarianism, that the relations of organism to environmental conditions were the same in the past as they are today. The more similar the fossil and modern organisms the more certain our interpretations can be. If we find a fossil leaf that bears detailed resemblance to leaves of modern beech trees, we identify the fossil leaf as that of the genus *Fagus*. We then use the climatic relations of the present-day species (about 9) to form a concept of those relations for the genus. With that information we attempt to reconstruct the climatic limits within which the tree of the past lived. It would be dangerous obviously to take one fossil beech leaf and reconstruct a beech forest; the modern beech forests in eastern North America, Europe, China, or Japan today may each have characteristics of composition and structure that are of relatively recent origin. Our study of necessity begins with the assumption that beech trees of the geologic past had ecologic requirements similar to those of beech trees today. We proceed by marshalling all available evidence into a logical order: first, evidence of flora and vegetation; then, in the context of the reconstructed vegetation, further evidence in two directions. In one direction we reconstruct the physical environment by comparing the vegetation evidence with evidence from sediments indicative of temperature regime, rainfall, etc. Particle size distribution, mineral composition, surface sculpturing, and many other characters can yield useful information. We work in the other direction by relating the vegetation evidence to the fossil record of animal life. For paleoecological interpretation of continental (as opposed to marine) deposits, vegetation is the key. The more evidence we disclose, the more we can revise and refine the ecological interpretaions, including those that add up to reconstructions of paleoclimates.

Long-Term Analysis of Atmospheric Pollen

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Abstract

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Detailed analysis of air-borne pollen has been carried on continuously by the University of Minnesota's Department of Botany since 1932. At that time a pilot operation was instituted after considerable observation of anemophilous species in the field. Pollen of many species from 50 different genera regularly occur each season on the Department's sampling slides. The ten most abundant kinds of air-borne pollen (arranged in order of decreasing importance) are: *Ambrosia* (ragweeds), *Artemisia* (wormwood-sage), Gramineae (grass), Chenopodiales (Russian thistle, pigweeds), *Rumex* (dock), *Quercus* (oaks), *Ulmus* (elms), *Betula* (birches), and *Urtica* (nettles). Such factors as precipitation, wind direction, and temperature, influence the record of pollen on the sampling slide. The date of the highest 24-hour concentration of any specific kind of pollen varies over the years. For example with ragweed pollen, the average date of highest 24-hour concentration was August 25; the earliest and latest maxima were observed, respectively, on Aug. 22, 1957 and Sept. 8, 1947. The range in total seasonal concentration of pollen (expressed as number of pollen grains per square centimeter of surface of the sampling slide) may vary extensively. With reference to ragweed pollen, a mean value of 3,300 pollen grains has been observed with a range of 1,324 (in 1962) to 6,530 pollen grains (in 1946).

Observations on pollen and spores in atmospheric space have been recorded since the middle of the 19th century when Blackley (2) and Airy (1) published their observations in England on pollen and spores, primarily with the prospect of their relationship to allergy. In this country beginning around 1916, Scheppegrell (16, 17), Duke and Durham (6, 7), Wodehouse (20, 21, 22), and others were instrumental in launching intensive regional studies of atmospheric pollen and spores which have been continued in a variety of ways to the present (see, e.g., 13, 14, 5, and 18).

Many different methods of sampling air-borne pollen and spores have been used. In a recent monograph, Dr. P. H. Gregory (12) has provided an excellent comparative account of various air sampling techniques. The relatively simple gravity sedimentation method has been widely used and, despite serious limitations, has provided much of the extant information on airborne pollen grains. As Hyde (13) observed from a comparison of his long-term studies using gravity methods with recent records obtained from application of the Hirst volumetric spore trap, the gravity slide is subject to the error of inadequately recording pollen and particles of smaller size, e.g., those having a diameter of 15 μ and less. In view of the many physical and biological variables involved in the incidence and behavior of airborne pollen and spores, quantitation and evaluation of these structures seem best conducted over a period of several years regardless of the sampling technique employed (13).

Our studies of the Twin Cities area were instituted in 1932 with daily records being obtained throughout all months of the first 4 years of the project. Note that some evidence for long-distance transport of pollen can be obtained from continuous sampling throughout the year (15, 10). Since 1936, most of our observations relate to the so-called growing season, March to October. Throughout, we have employed the gravity sedimentation method. Standard microscope slides (3 x 1 inch) were aseptically surfaced with an oil preparation[1] devised to provide favorable receptivity, surface continuity, and optical properties. These slides were exposed horizontally to the atmosphere at a height of ca. 70 ft above ground for 24 hours in the pollen slide shelter designed by Durham (8, see also 4).

[1] The medium devised by us for entrapping airborne particulates (including pollen, spores, etc.) consists of: 2 parts, melted white petrolatum; 1 part, paraffin (mineral) oil; 1 part, *n*-butanol; 1 part, xylene. Stir while warm to insure a uniform mixture. The mixture is kept free from contamination in a stoppered container. A uniform long-lasting coating on the sampling slides is easily established with a glass applicator rod. Exposed slides, when protected from contamination, will keep indefinitely.

Four sampling stations, including one in a residential section approximately five miles from the three University stations, have been used. In earlier phases of the project, sampling slides were exposed in Duluth, Moorhead, Montevideo, and Pipestone, Minn.; LaCrosse and Madison, Wisc.; Des Moines, Iowa; Wheaton, Ill., and Springfield, Mo. (see 15).

Evaluation of the kinds and quantities of pollen and spores is based on the analysis of 25 microscopic fields (100 x) systematically distributed across the width of the slide in five strips (15). The pollen-spore data thus obtained were expressed in terms of number of individual pollen grains per square centimeter of surface of the exposed slide. These data provided the basis for qualitative and quantitative interpretation of atmospheric pollution during the period for which the sampling slide was exposed. Since pollen grains are commonly not the smooth, solitary spheres of Stokes' formula, we have preferred to omit Stokesian calculations which, in theory, are designed to yield data indicating concentrations on a volumetric basis (see 3).

In size, pollen grains of all taxa observed on the exposed slides range from ca. 14 μ to 110 μ in diameter. Pollen types occurring in greatest abundance fall within the range, 20 to 60μ.

The kinds and amounts of pollen grains and spores vary with the seasons and with the geographical location. In addition, from one year to the next, variables such as: amount and period of precipitation, occurrence of unduly late spring, as well as precocious autumn frosts, and man's disturbance of the vegetational cover will influence the atmospheric pollen record (Table II).

Consequently in theory, there is much that can be read in the long-term record of atmospheric pollen (18).

With reference to this geographical region, pollen representing approximately 50 different genera occur regularly each year on the sampling slides (Fig. 1). The average total number of pollen grains per square centimeter for the season is approximately 7,400 (Table I). During the last 10 years, the total amount of ragweed (*Ambrosia, Iva*) pollen has fallen well below the 20-year average of ca. 3,300 grains per square centimeter (Fig. 2, Table II). With this group, the date of maximum atmospheric concentration can be expected during the last week of August (Fig. 3). Commonly, the maximum 24-hour count of ragweed pollen is below 400 grains per square centimeter (Figs. 4, 5). Pollen of such other groups as the grass (*Gramineae*), oak (*Fagaceae*), elm (*Ulmaceae*), and chenopod (*Chenopodiaceae, Amaranthaceae*) families have retained relative prominence for the last 15 years (Fig. 1).

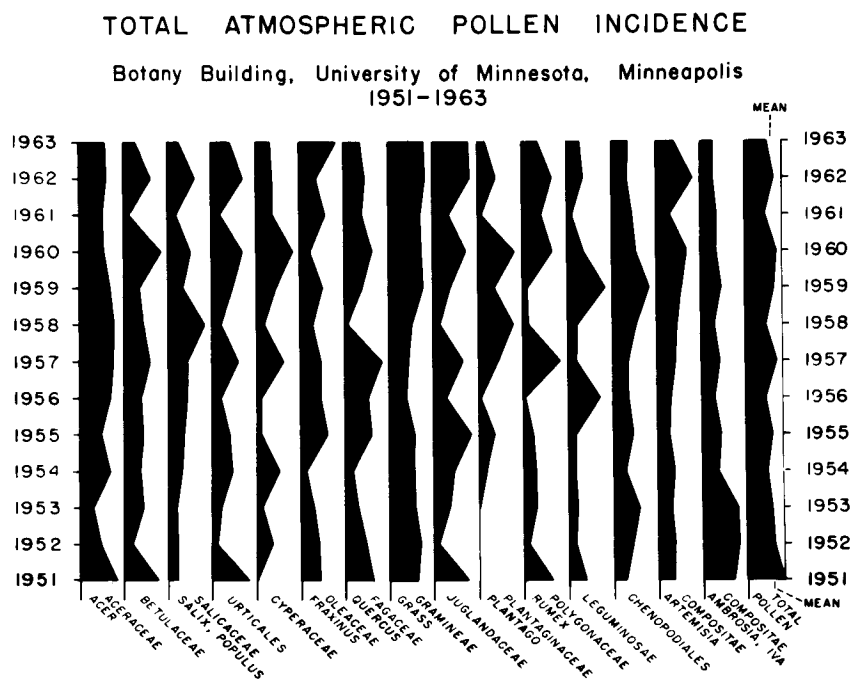


Fig. 1. Total annual incidence of atmospheric pollen of all taxa for entire growing season, March-October, 1951-1963. Quantitation is on the basis of number of pollen grains/cm^2 of sampling surface. Abscissae for individual pollen groups represent number of pollen grains/cm^2 of sampling surface. Actual quantitation is recorded in Tables I and II.

Table I

Total Atmospheric Pollen Incidence, 1951-1963*

Year	Botanical Group																
	Aceraceae *Acer*	Betulaceae	Salicaceae *Salix, Populus*	Urticales	Cyperaceae	Oleaceae *Fraxinus*	Fagaceae *Quercus*	Gramineae Grass	Juglandaceae	Plantaginaceae *Plantago*	Polygonaceae *Rumex*	Leguminosae	Chenopodiales	Compositae *Artemisia*	Compositae *Ambrosia, Iva*	Miscellaneous**	Total Pollen
1963	332	105	372	1,888	31	269	704	429	55	6	13	31	288	70	1,369	311	6,273
1962	347	246	917	3,211	36	126	899	430	56	14	24	35	257	140	1,333	268	8,339
1961	284	54	324	891	37	185	768	365	24	3	15	13	353	53	1,780	414	5,563
1960	309	329	743	3,051	76	76	1,197	384	50	28	24	35	398	114	1,703	207	8,724
1959	391	144	508	2,168	42	162	769	396	26	13	4	84	619	94	2,311	324	8,055
1958	443	177	1,210	975	16	85	137	260	11	26	5	22	404	74	1,517	283	5,645
1957	429	233	649	2,590	57	148	1,572	226	44	15	30	19	271	73	2,056	279	8,691
1956	386	162	635	915	11	146	1,007	201	20	2	2	73	273	55	1,492	130	5,510
1955	274	170	543	1,804	11	194	1,130	290	56	12	8	18	355	47	2,164	232	7,308
1954	378	141	475	2,018	48	38	346	286	30	6	10	16	207	67	1,871	279	6,216
1953	158	174	305	938	14	97	596	302	23	...	10	21	440	55	3,923	202	7,258
1952	259	73	331	578	32	133	860	351	8	...	4	17	331	64	4,041	402	7,484
1951	470	313	341	3,676	...	139	1,159	333	51	...	23	41	202	58	3,332	300	10,438
Total	4,460	2,321	7,353	24,703	411	1,798	11,144	4,253	454	125	172	425	4,398	964	28,892	3,631	95,504
Mean	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	7,346

*Botany Building, University of Minnesota, Minneapolis. Pollen data are expressed as number/cm^2 of sampling surface.
**Includes such taxa as: basswood, linden (Tiliaceae, *Tilia*; conifers (Pinacea); cat-tail (Typhaceae, *Typha*), and grape (Vitaceae).

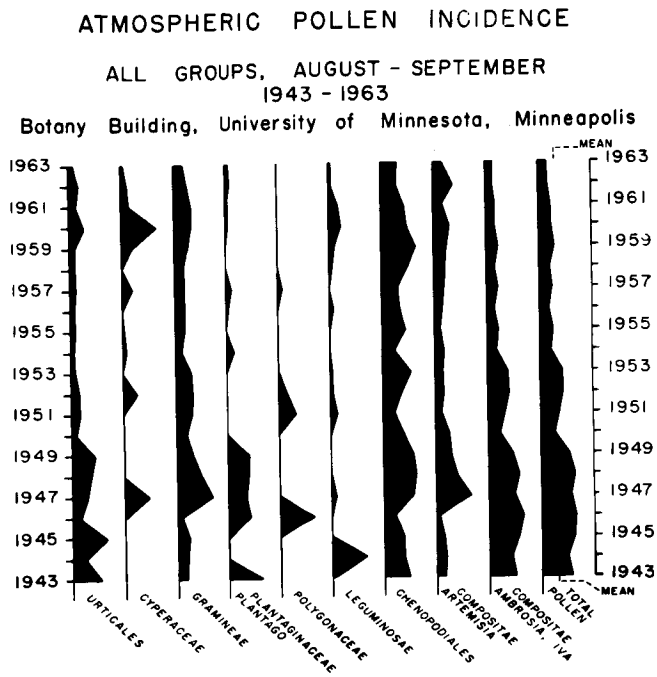


Fig. 2. Total concentration of airborne pollen during August-September, 1943-1963. Quantitation as in Fig. 1.

Note that on the basis of extensive clinical observations (9) seven of the ten most abundant pollens in the atmosphere are prominent as major causes of pollen allergy. Arranged essentially in order of clinical importance these pollen groups are: ragweed, wormwood (*Artemisia*), grass, chenopod, dock (*Rumex*), oak (*Quercus*), and elm (*Ulmus*), nettle (*Urtica*).

With possible reference to paleoecology, data on atmospheric pollen can be used to sense the validity of predicting the major features of the total vegetational landscape primarily on the basis of the pollen record (as it is done in palynological investigations based on the nature of the fossil pollen occurring in various sediments) (11). For this region, the atmospheric pollen slides provide evidence for a vegetational community in which ragweed (Compositae, *Ambrosia*); elm, nettle (Urticales); oak (Fagaceae, *Quercus*); poplar, willow (Salicaceae); maple, box elder (Aceraceae, *Acer*); chenopods (Chenopodiales); grass (Gramineae); birch (Betulaceae); ash (Oleaceae, *Fraxinus*), and wormwood (Compositae, *Artemisia*) have prominence. These ten components have been arranged in order of decreasing pollen incidence on the atmospheric slides. As one would expect, the sampling slides have given virtually no evidence for the presence of the many insect-pollinated species which together comprise an important element in the current, vegetational community.

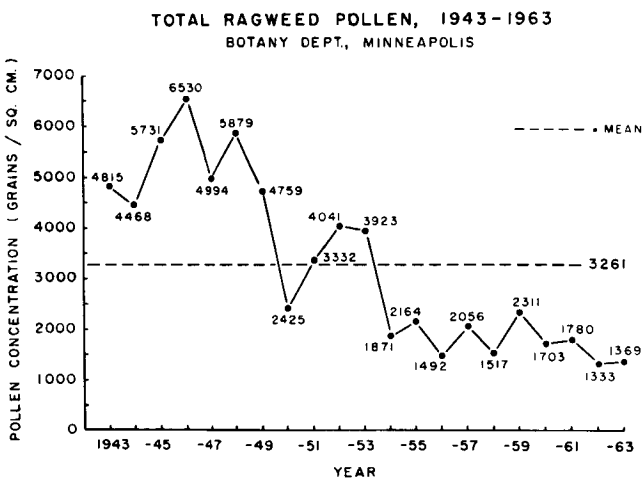
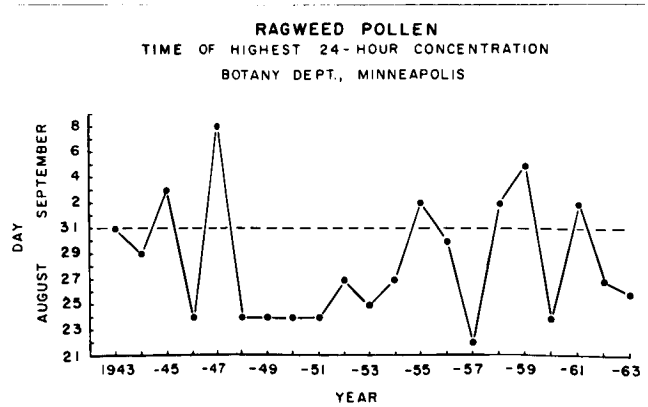


Fig. 3 (*top*). Total annual concentration of ragweed pollen, 1943-1963.

Fig. 4 (*bottom, left*). Date of highest daily concentration of ragweed pollen, 1943-1963.

Fig. 5 (*bottom, right*). Highest daily concentration of ragweed pollen for each of the growing seasons, 1943-1963.



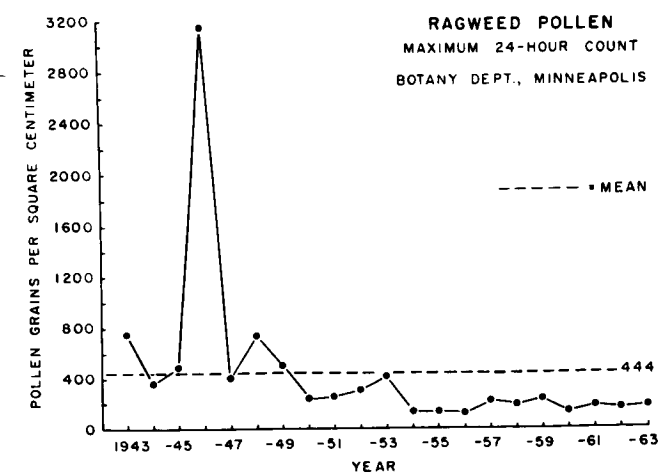


Table II

Atmospheric Pollen Incidence
All botanical groups, August-September, 1943-1963*

Year	Botanical group										Meteorological data (19)			
	Urticales	Cyperaceae	Gramineae	Plantaginaceae	Polygonaceae	Leguminosae	Chenopodiales	Compositae *Artemisia*	Compositae *Ambrosia, Iva*	Miscellaneous	Total Pollen	Avg. temp., °F		Pptn., inches	
												Aug.	Sept.	Aug.	Sept.
1963	52	1	67	3	...	2	236	60	1,336	6	1,763	68.9	62.2	1.55	3.47
1962	96	3	89	3	...	2	217	132	1,324	4	1,870	68.3	56.4	3.47	2.46
1961	70	4	111	2	...	8	332	53	1,779	5	2,364	71.3	59.1	2.38	3.01
1960	145	18	111	2	...	11	370	109	1,697	1	2,464	72.1	61.8	3.99	3.79
1959	59	5	93	...	...	5	498	91	2,296	9	3,056	75.2	63.1	6.60	2.29
1958	61	...	61	...	...	3	385	72	1,512	30	2,124	71.4	62.7	3.03	1.09
1957	59	5	68	5	1	...	230	70	2,048	5	2,491	70.9	59.4	5.75	1.65
1956	51	...	67	2	...	4	260	55	1,492	10	1,941	71.2	58.7	5.22	0.79
1955	57	1	64	...	...	...	342	42	2,152	6	2,664	76.1	63.0	2.84	0.99
1954	39	2	40	6	...	...	179	67	1,845	8	2,186	70.4	60.5	3.08	3.65
1953	53	...	106	...	...	2	419	55	3,915	5	4,555	73.3	62.1	2.75	0.55
1952	81	7	111	...	2	2	279	61	4,026	4	4,573	69.1	63.1	4.18	0.42
1951	82	...	108	...	4	6	183	46	3,329	20	3,778	68.1	56.9	1.94	5.80
1950	56	...	70	...	...	...	301	90	2,418	10	2,945	67.7	62.6	1.84	1.46
1949	224	...	111	4	...	...	424	107	4,740	8	5,618	74.2	58.4	2.64	2.67
1948	190	...	167	17	...	...	465	140	5,856	...	6,835	72.7	67.8	3.37	1.04
1947	146	12	240	15	...	4	427	234	4,988	25	6,091	78.2	63.1	2.41	1.48
1946	88	...	29	19	8	...	156	25	6,530	...	6,855	68.8	59.8	0.43	6.58
1945	347	...	76	...	...	...	293	54	5,737	...	6,507	71.0	60.2	2.27	2.13
1944	152	...	71	...	...	31	293	68	4,496	...	5,111	71.6	61.6	3.65	0.97
1943	306	...	62	28	...	2	352	57	5,069	...	5,876	71.9	58.2	1.75	2.47
Mean	...	...	...	...	...	...	...	...	3,266	...	3,889				

*Data on pollen expressed as number/cm^2 of sampling surface. Botany Building, University of Minnesota, Minneapolis.

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Discussion

Belmont — Have you any explanation for the decrease in overall pollen activity in recent years? Have you made any comparisons with weather at the time?

Dahl — We are examining these details with reference to the detailed weather. In recent times we do have the recording of cooler than average seasons. In the case of some of these groups one also has to examine the factor of man's disturbance of the vegetational cover. During the last 20 years there has been what could be considered to be an improvement in the sense of eliminating weedy areas. Our collection slides are exposed on top of the Botany Building here in Minneapolis. This area is above the Mississippi River channel. Some of the great expanses of weedy areas along the banks of the Mississippi have been removed. This action is reflected in the pollen records.

Benninghoff — Have you any explanation for the changes in relative amounts of grass and chenopod pollen over the last 15 years?

Dahl — This is not an easy question to answer. The chenopod grass situation is sensitive to a number of factors. This does not relate, Dr. Benninghoff, directly to your question, but I think you may be interested in this observation. During the unusually dry years in the mid 1930's, areas that had been covered by grass (this would be reflected in the pollen count) were eliminated by extreme dryness. This created new areas for Russian thistle, a member of the chenopod group. If one were to total the grass pollens (and we have done this) he would see that the grass pollen total decreased while the chenopod total increased. There were great areas opened to the chenopod group.

Now in terms of the record of the recent years you saw an increase in both grasses and chenopods. The increase in grass seems to be related to a reasonably favorable annual precipitation. The increase in the chenopod group cannot be explained quite so easily. This explanation does not check with the Russian-thistle group, but there may be other components of the Chenopodiales involved here. I am sure Dr. Benninghoff is familiar with the fact that this particular group, the Chenopodiales, is unfortunately or fortunately, depending upon your problem, favored by a uniform pollen structure. In other words, it is difficult to tell all genera of the Chenopodiaceae from all genera of the Amaranthaceae, so that one ordinarily lumps these two families together and records them as pollen of the chenopods. The various members of these two families differ in their ecological requirements. This is why it is unfortunate that we cannot track this down. Possibly, Dr. Benninghoff, with reference to the increase in the chenopods, you do have an increase in some members which also tolerate the same growing conditions as do the grasses.

Airplane Trapping of Organisms and Particles

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Abstract

An air plankton trap for operation on a Super Constellation aircraft was developed in 1960 by Bishop Museum, Honolulu, Hawaii. During 3 years of operation 59 arthropods plus numerous fragments have been collected over the Antarctic, Pacific, and continental United States. Plant and mineral material have also been collected and analyzed. The trap has been operated about 668,500 km (415,260 statute miles).

Collecting results and in-flight tests indicated low efficiency. Wind tunnel tests by Lockheed show that modifications are necessary. The trap is awaiting proposed changes to improve insect collecting and to add devices for other disciplines.

Introduction

The Entomology Department of the Bishop Museum has extended its scope across the Pacific, into Southeast Asia, and to the Antarctic continent. Though the prime object has always been to collect, preserve, and classify as many specimens as possible, such a collection is more meaningful when coupled and supplemented with a program to study zoogeography and evolution. If knowledge of the origins, development, and relationships of the insect fauna of the oceanic Pacific islands is to be complete, a study of dispersal across oceans becomes necessary. Such a study was initiated in 1957 when screen traps and later cone shaped nets were used for trapping aboard ships in the tropical Pacific (2,7,8,9).

In 1959 studies of airborne organisms in the Antarctic area were begun on land, at sea, and in the air from Otter planes (3,5). Collecting aboard ships throughout the Pacific has been improved and continued (1,10,11,12).

Since altitude and wind currents are prime factors in the dissemination of arthropods between land masses, a proposal for a high speed airplane trap was submitted early in 1960. Both the U. S. Antarctic Research Program and the National Science Foundation backed this new program financially. With the cooperation of the U. S. Navy and Lockheed Aircraft Corp. a trap was completed in September 1960 for use in a Super-Constellation airplane. The trap has been operated during Operation Deep Freeze 1961, 1962, and 1963 (4,11).

Description

The trap (Figs. 1-3) was an all metal duct 595 cm long. When mounted on the starboard side of an aircraft the forward opening is held firmly in place about 15 cm from the fuselage just aft of the cockpit hatch — well forward of the wing and propellers. To avoid cutting a separate set of holes in the fuselage of the aircraft, the foreward two porthole windows were removed and used as points of entrance and exit.

Since the air speed had to be reduced from more than 200 knots to less than 25, the duct gradually increased in diameter: 10 cm at the entrance, 25 cm where it passed through the porthole opening, and 45 cm inside the cabin of the aircraft. After being filtered by a funnel-shaped fine-meshed stainless steel screen that lined the 45 cm portion, the trap began to decrease in size, with a diameter of 25 cm at the second porthole and about 15 cm at the exhaust opening.

The collecting receptacle was a small, screen metal cup. This removable cup at the apex of the screen funnel was 365 cm from the intake opening.

Two valves, one anteriorly and one posteriorly, in the cabin portion of the duct prevented passage of air while the trap was not being operated. During sampling at high altitudes this section could be sealed off and depressurized. This permitted an air tight door in the side of the enlarged cylinder to be opened and the collecting receptacle with sample replaced as often as necessary. Fig. 1 is an early draft of the plan; several modifications were made before the trap was completed.

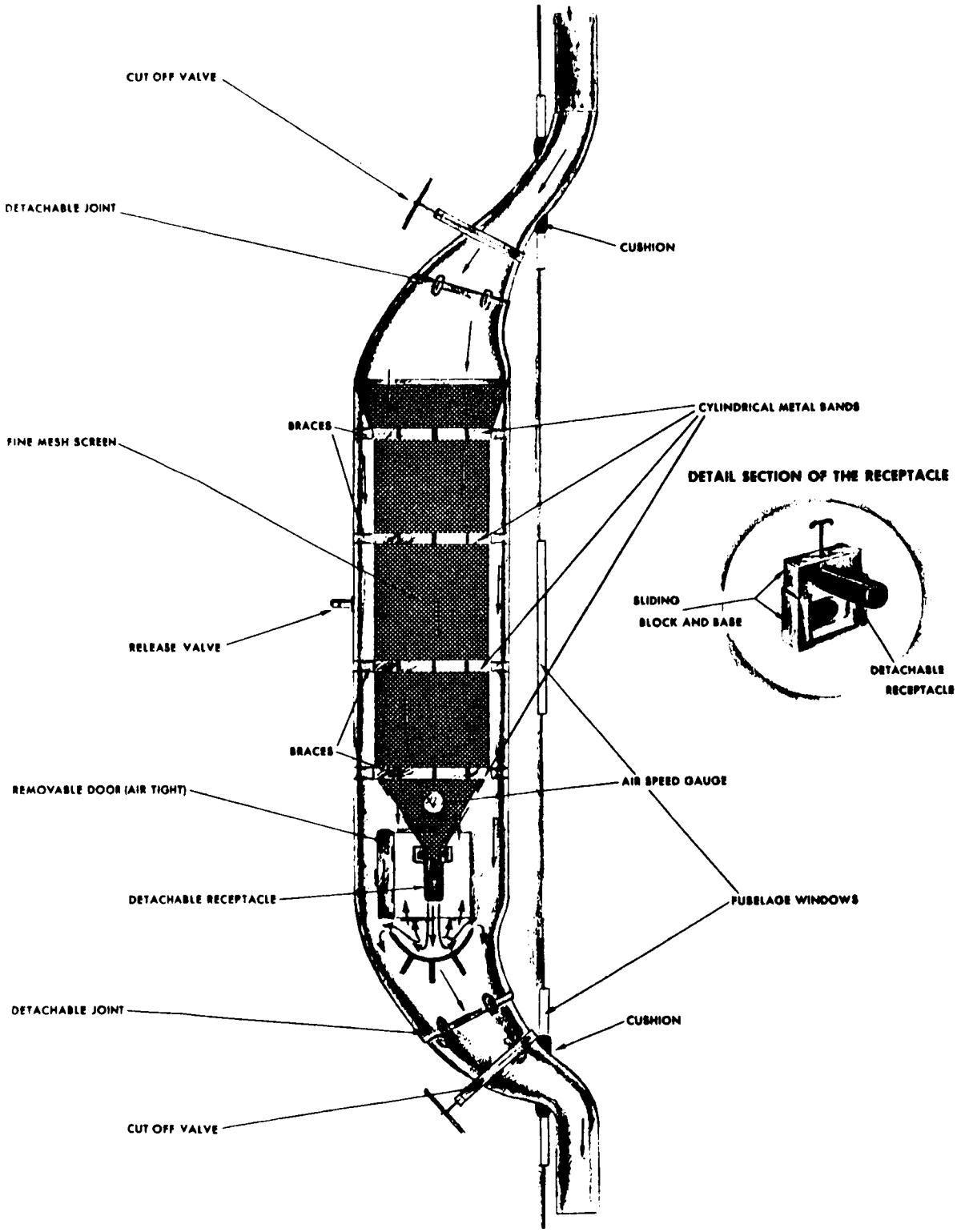

Fig. 1. Preliminary diagram of internal airplane trap. Actual trap varies in several aspects.

The trap was operational after a deicer around the intake opening was added.

Methods

An entomologist from the Museum accompanied each sampling flight. When possible the collecting receptacle was replaced at about every 1,000-meter altitude change and at regular intervals while cruising. The receptacle was immediately checked and all samples transferred to small vials that were later sent to Honolulu for microscopic check at the Museum.

At the end of the first year of operation other organizations became interested in our program. The Astrophysical Observatory in Cambridge, Mass., cooperated by analyzing the extraterrestrial particles. The only modification in previous collecting techniques for this new phase in the program was to sweep all particles from the receptacle with a camel hair brush. As before, these samples were checked microscopically at the Museum for insects and then forwarded to the Observatory for detailed chemical analysis by the electron-beam microanalyzer technique (6).

The Geochronology Laboratories of the University of Arizona in Tucson, Ariz., also became an active participant during the second year. Microscope slides with glycerine jelly and stain (fucsin) were prepared at the University of Canterbury in Christchurch, New Zealand and exposed during flight to study dispersal of pollen at high altitudes.

At first the slides were fastened by masking tape to a baffle directly behind the collecting receptacle; a clamp was later used to facilitate exposing the slides. The exposed slides were sealed with cover glasses and shipped together with data sheets to Tucson for microscopic examination.

Operation

The trap was first mounted in November 1960 on a Super Constellation at Christchurch, New Zealand. It was in operation on this plane for the past three Antarctic seasons (1961, 1962, and 1963); during the "off seasons" (1961 and 1962) it was transferred to a Super Constellation for operation between the island of Hawaii and Midway Atoll. To date it has been operated about 668,500 km (415,260 statute miles) including the following:

1. Antarctic season; November 1960 to March 1961; 187,900 km.
   a. Five return trips between Christchurch, New Zealand and Quonset Point, R. I. via Honolulu, Hawaii; 161,000 km (100,000 statute miles).
   b. Two return trips between Christchurch and McMurdo Sound, Antarctia; 13,600 km (8,424 statute miles).
   c. Post-Antarctic season; one return flight Oahu Island — Hawaii Island and three return flights Oahu-Midway Atoll; total 13,300 km (8,260 statute miles) (4).
2. Antarctic season; September 1961 to April 1962.
   a. Between Quonset Point, R.I. and McMurdo Sound, Antarctica via Honolulu, Hawaii and Christchurch, New Zealand; 197,300 km (122,577 statute miles).
3. Post Antarctic season; May to August 1962.
   a. Between Island of Hawaii and Midway Atoll (11); 19,310 km (12,000 statute miles).
4. Antarctic season; September 1962 to April 1963; 264,000 km (164,000 statute miles).
   a. Two return trips between Rhode Island and New Zealand via Hawaii; 64,400 km (40,000 statute miles).
   b. 23 return trips plus 3 turn-around trips (due to engine trouble and/or bad weather) between New Zealand and McMurdo; 188,300 km (117,000 statute miles).
   c. Internal New Zealand flights plus one return flight to Australia; 11,300 km (7,000 statute miles).

Results

Air from sea level to 5,790 meters (19,000 ft) has been screened. Insects collected to date are listed in Tables I to II; IV to VI. 1) In Polar Zones all courses and directions are given in grid coordinates, in all tables in this report these have been converted to true. 2) On the flights between Oahu and Midway Atoll insects were taken only when the plane flew on the north side of the island chain and when the wind was from the southwest (after turning around over the main Hawaiian Islands). 3) Between Canton and the Hawaiian Islands, a lygaeid bug was taken at an altitude of 4,960 meters — the nearest land was Johnston Island approximately 248 km away.

Williams Field on McMurdo Sound is about 77° 30'S latitude and 165° 50'E longitude. Several interesting catches were made near the Antarctic continent. A wing of a lygaeid bug, probably of *Nysius huttoni*, was caught at 4,000 meters (66° 59'S lat). An Ichneumonid wasp, *Diplazar laetatorius* Fabricius ♀ was removed from the trap at 3,353 meters (72° 04'S lat; 169° 40'E long). Some question may be raised on the validity of this latter catch for on several occasions insect parts were recovered several checks before and several after the main fragment was retrieved.

During the first year of operation (1960-1961) the vegetable and mineral material taken in the trap was analyzed at the Museum (Table III). The second year (1961-1962) the Museum only recorded insects. All mineral samples were sent to the Smithsonian Astrophysical Observatory where some of the particles were subjected to detailed chemical analysis by the electron beam microanalyzer technique.

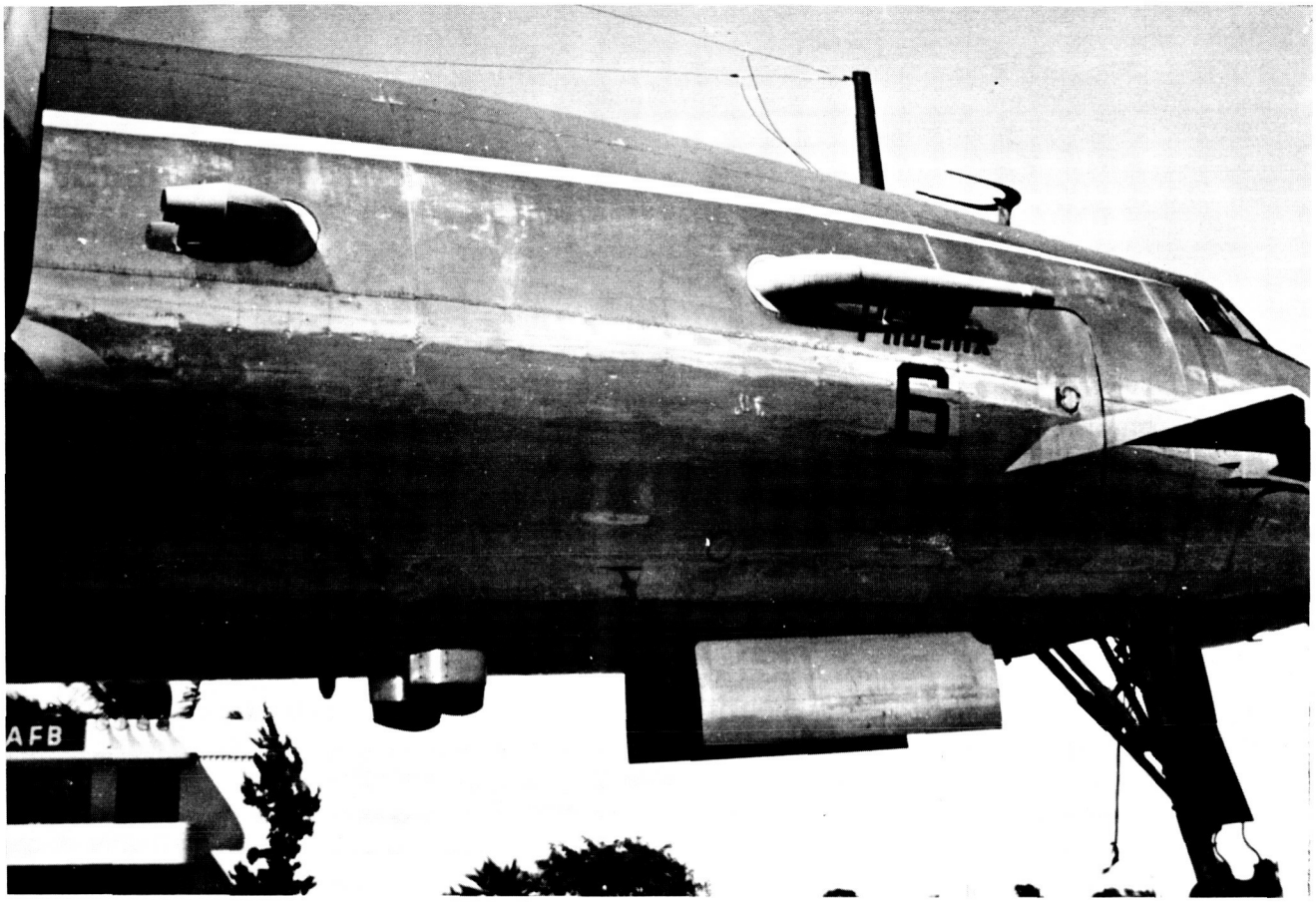

Fig. 2. Forward part of U.S. Navy aircraft C-121J BUNO 131624, showing trap inlet and outlet (Official U.S. Navy photograph).

The results of the analyses of these samples from the Antarctic atmosphere became part of a report on representative particles collected from a number of other localities, including 750-year-old Greenland ice, 55-year-old Antarctic ice, glacial ice caves, a New Mexico mountain top, and the stratosphere. A total of 118 representative particles from the sources listed above were divided into two main classes: spherules and irregulars. The samples reported by the Bishop Museum, collected at 13,000 ft about 70° south latitude and 171° east longitude, contained no spherules. One of the irregular particles was rich in silicon, indicating it may have been an ablation of a stony meteorite-type body and hence of cosmic origin.

In the original publication the remaining particles analyzed from the Antarctic atmosphere were placed in the table as "Other Irregular Particles." One analyzed was rich in chromium and silicon. All in the "Other Irregular Particles" category contained aluminum. Due to the composition of the particles from this trapping program, they cannot be definitely classified as terrestrial or extraterrestrial (6).

The glycerine jelly air slides which were exposed during the 2nd and 3rd years were sent to Dr. Lucy M. Cranwell of the Geochronology Laboratories, University of Arizona, Tucson, for examination. Dr. Cranwell is making a comparative study of fossil pollen and spores from younger Antarctic deposits and has been interested to check flights of organic material from countries to the north of Antarctica (personal communication). Some of the results of the work will be given Aug. 5, 1964, by her in the Aerobiology section of the Tenth International Botanical Congress in Edinburgh. Particular reference will be made to the recovery of very small amounts of *Nothofagus fusca*-type pollen (8-pored) and of small fern spores as far south as the approaches to McMurdo Sound. Pollen grains of *Nothofagus* increased in frequency near the main New Zealand islands, while pine pollen (of species introduced to New Zealand) were recovered in abundance above Canterbury on October 23/24, 1961. Other types found included pollen of podocarps, caryophylls, grasses, *Coprosma*, chenopods; both monolete and trilete spores of ferns; varied fungal spores and fragments, together with much comminuted waxy material (including hairs of plants and broken spores). During some periods much

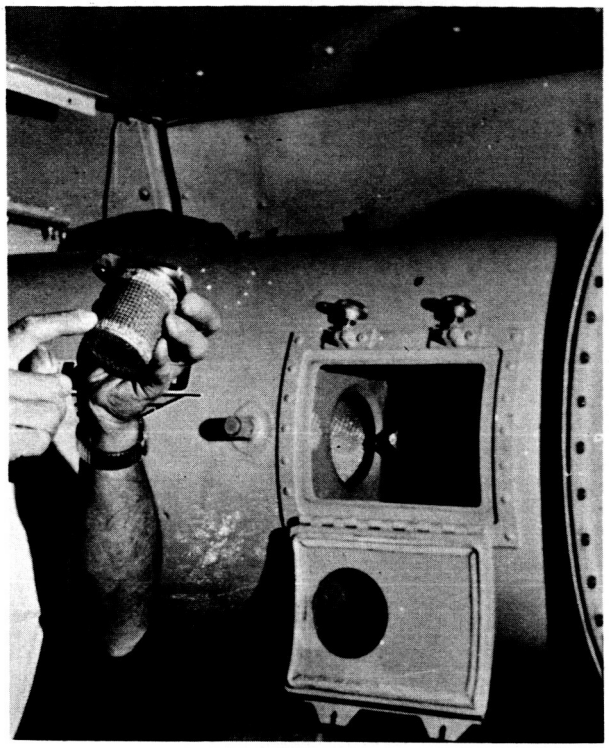

Fig. 3. Trap in operation; collecting receptacle removed for examination (Official U. S. Navy photograph).

mineral material, together with other soil particles was picked up in the vicinity of New Zealand.

A detailed report is being prepared, but the work of identification is slow.

The Department of Oceanography and Meteorology of Texas A. & M. University has already been sent samples from the Museum's Air Trapping program. Members of that department are presently working up the material and will relate it to their program of air pollution.

Attempts were made during the 1963 Antarctic season to collect viable microorganisms. Though no valid information resulted, collecting techniques were developed that might be useful in the future.

The results of wind tunnel tests by Lockheed in November 1963 are given in Figures 4 and 5 and Table VII. As originally calculated, the air flow through the collecting receptacle was to be about 20 knots. Wind tunnel tests 1 A & 1 B show that the velocity at that point was actually about 2.5 knots. Further tests proved that the fine-meshed screen prevented air from flowing as calculated.

Modifications will increase the wind velocity to an optimum (ca. 15 knots). The metal duct should not be altered though the valves will be changed. Filtering devices will be added for the collection of salts, microorganisms, and/or other particles from the atmosphere.

Table I

Insects Taken in High Speed Trap on Antarctic Program

Date	Wind		Plane speed, knots	Lat	Long	Alt,† meters	Order	Family	Trapper
	Direction	Veloc., knots							
1960									
20, XII	320° - 290°	10-12	223	31° 35' - 27° 42'S	176° 30' - 178° 00'E	3,350	*Diptera	Sciaridae	S**
XII	290°	12°	223	27° 42'S	178° 00'E	3,350	*Diptera	Chironomidae	S
1961									
5, III	340°	20	180	Christchurch to 42° 00'S	172° 30'E	10-2,130	*Diptera	Syrphidae	W
6, III	265°	20	255	29° 00'- 32° 10'N	146° 30'- 138° 00'W	5,790	*Isoptera	?	W
7, III	270°	20	245	38° 45'N to Washington, D. C.	76° 55'W	1,830-10	‡ Hymenoptera	Encyrtidae?	W
7, III	270°	20	245	Washington, D. C. to 38° 48'N	76° 36'W	10-2,740	‡ Hymenoptera	Scelionidae (alive)	W
10, III	270°	10	200	Alameda, Calif. to 37° 40'N	122° 30'W	5-1,520	*Diptera	?	W
12, III	250°	20	220	3° 44'- 10° 20'S	172° 05'- 174° 21'W	1,830	*(Arthropoda)	?	W

‡ Taken over North American continent
*Incomplete specimen
**Sedlacek, S; Wise, W.
† Single entries are at cruising altitude

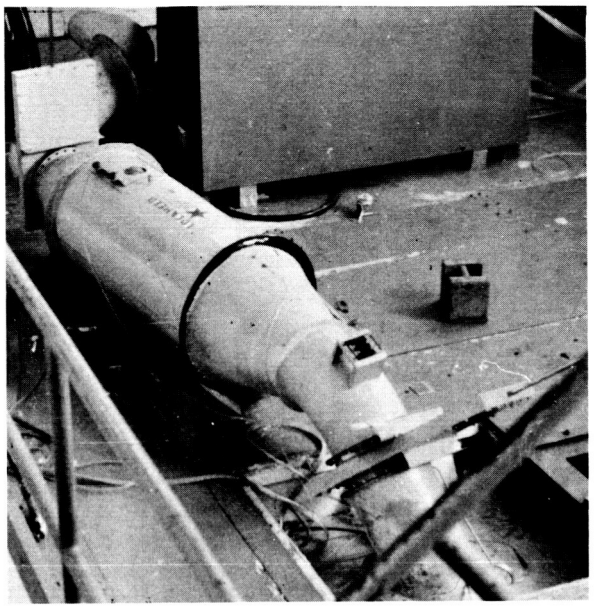

Fig. 4. Trap set up for wind-tunnel tests (Lockheed Aircraft Corp. photograph).

Table II

Insects Taken in High Speed Trap Between Hawaii and Midway Atoll (Yoshimoto)

Date	Wind		Plane speed, knots	Lat	Long	Alt, † meters	Order	Family
	Direction	Veloc., knots						
1961								
5, V	225°	28	240	Oahu to 21° 25'N	158° 20'W	1, 830	Heteroptera	Lygaeidae: *Nysius*
5, V	225°	28	240	"	"	1, 830	*Heteroptera	Lygaeidae: *Nysius*
5, V	225°	28	240	21° 52'-22° 05'N	159° 30'-160° 07'W	3, 050	Acarina:Trombidiiformes	
5, V	225°	28	240	25° 15'-26° 28'N	168° 40'-171° 50'W	3, 050	*Heteroptera *Diptera	Lygaeidae: *Nysius*?
5, V	225°	28	240	26° 28'-27° 40'N	171° 50'-175° 32'W	1, 980	*Lepidoptera Heteroptera	Lygaeidae: *Nysius*
5, V	225°	28	240	27° 40'N	175° 32'W	1, 980	Heteroptera	Lygaeid nymph 1st instar
5, V	210°	25	250	28° 10'N	177° 20'W	3, 050	*Heteroptera	Lygaeidae: *Nysius*
5, V	210°	25	250	22° 15'N	160° 14'W	3, 350	*Coleoptera	(Small beetle)
22, V	290°	25	250	23° 45-23° 10'N	164° 55'-163° 05'W	3, 960-2, 740	*Heteroptera	Lygaeidae
29, VI	242°	15	210	Oahu to 21° 22'N	158° 19'W	1, 800	*(Insect)	(Fore wing)
29, VI	242°	15	210	21° 22'-21° 00'N	158° 19'-159° 00'W	2, 440	Psocoptera	Pachytroctidae: *Psyllonera williamsii* Banks?
29, VI	242°	15	210	21° 00'-22° 05'N	159° 00'-160° 00'W	2, 440	*Psocoptera	
29, VI	242°	15	210	22° 30'-23° 14'N	161° 00'-163° 00'W	2, 590	Homoptera	Exuviae of 1st instar nymph of Cicadellidae

*Incomplete specimen
†Single entries are at cruising altitude

Table III

Vegetable and Mineral Material Trapped (Selected Samples)

Date	Wind: Direction	Wind: Veloc., knots	Plane speed, knots	Lat	Long	Alt†, meters	Material	Trapper
1960								
20, XI	230°	28	180	73° 02'S	172° 40'E	1,800	Mineral: granite 1.7 x 1.5 x 1 mm	S**
2, XII	130°-210°	12	225	17° 30'-11° 30'N	159° 20'-162° 40'W	3,000	Mineral: limestone, etc.	S
5, XII	170°-250°	12-45	220	61° 55'-59° 20'S	167° 20'-168° 10'E	3,050	Mineral: granite 0.5 x 0.7 mm	S
14, XII	125°	55	248-260	49° 45'-45° 00'S	174° 30'-173° 30'E	1,800-3,000	Plant: monocot leaf sheath	S
1961								
27, I	250°	13	190	37° 40'-36° 35'N	122° 47'-125° 05'W	1,200	Plant: fragments	S
30, I	250°	25	215	32° 10'-36° 30'S	176° 20'-174° 45'E	1,200-1,800	Plant: fragments	S
21, II	250°	25-38	244	59° 50'-55° 40'S	167° 30'-169° 45'E	3,050	Mineral: granite 5 x 4 x 2.5 mm	
6, III	270°	12	253	32° 10'N	138° 00'W	5,790	Plant: spines, scales	S
9, III	270°	20	240	41° 33'N	73° 52'W	3,050	Mineral: sand-quartz & igneous?	W
9, III	270°	20	240	41° 43'N	79° 25'W	3,050	Plant: algae?	W
10, III	290°	20	230	32° 47'N	134° 25'W	2,400	Plant: stem	W
25, III	270°	10	216	41° 22'N	81° 43'W	2,400	Plant: fragments	W
5, V	240°	25	210	22° 05'-24° 40'N	160° 07'-166° 55'W	3,050	Mineral: sand	Y
5, V	240°	25	210	26° 28'-27° 40'N	171° 50'-175° 32'W	3,050	Mineral: sand	Y
6, V	235°	30	210	28° 12'-28° 10'N	177° 23'-177° 20'W	2,400	Mineral: sand	Y
29, VI	242°	15	210	Oahu to-21° 22'N	158° 19'W	5-1,800	Plant: stem	Y
29, VI	242°	15	210	21° 22'-21° 00'N	158° 19'-159° 00'W	2,400	Plant: *Eufilicales sporangium*	Y

**Sedlacek, S; Wise, W; Yoshimoto, Y.
† Single entries are at cruising altitude

Table IV

Insects Taken in High Speed Trap on Antarctic Program (Mitchell)

Date	Wind direction, velocity, knots	Plane speed, knots	Lat Long	Alt, † meters	Order	Family	No. specimen
1961							
30, VIII			Takeoff from Quonset Point, R. I.	0-620	Dipt.	Muscoid fly	1
30, VIII			40° 50'N 72° 55'W 39° 50'N 74° 25'W	3,100	Lepidopt.	Leg	1
30, VIII			Landing approach Andrews Field, Wash., D. C.	465-0	Lepidopt.	Leg	1
8, IX			38° 05'N 121° 55'W 38° 25'N 121° 40'W	930-2,170	Hymenopt.	Agaontidae	1
25, X	330° /20	185	43° 30'S 172° 10'E 42° 30'S 172° 00'E	3,860	Hemipt.	Lygaeidae?	1
25, X	220° /13	190	29° 15'S 177° 15'E 27° 25'S 177° 55'E	3,810-5,270	Coleopt.	Curculionidae	1
25, X	115° /12	185	04° 55'N 168° 05'W 14° 10'N 160° 40'W	4,960	Hemipt.	Lygaeidae?	1
29, XI	80° /20	200	66° 00'S 171° 00'E 59° 37'S 170° 20'E	4,000	Hemipt.	Lygaeidae: *Nysius huttoni?*	1
11, XII	110° /25	220	Takeoff Christchurch, N. Z.	0-620	Thysanopt.	Thripidae	2*
11, XII	110° /25	185	44° 40'S 171° 30'E	2,144-2,480	Thysanopt.	Thripidae	1
15, XII			Takeoff Hickam AFB	0-620	Dipt.	Ceratopogonidae: (*Forcipomyia ingrami* Carter ♂)	1
1962							
24, I			Harewood vicinity, Christchurch, N. Z.	930-2,697	Homopt.	Aphididae	1
28, I			Takeoff Christchurch, N. Z.	0-620	Hymenopt.	Eulophidae	1*
28, I	120° /40	170	44° 00'S 172° 00'E 44° 30'S 171° 45'E	3,215-3,100	Coleopt.		1*
2, II			Takeoff pattern, Chch., 43° 23'S 172° 11'E	620-2,170	Hymenopt.	Braconidae	1*
2, II	80° /10		43° 55'S 172° 11'E 44° 23'S 171° 49'E	2,325-3,255	Psocopt.	Mesopsocidae	1*
19, II	140° /15	195	19° 55'S 177° 00'E Touchdown Fiji	3,286-0	Dipt.	Ephydridae	1
20, II	100° /8	177	Takeoff pattern Fiji 18° 50'S 177° 15'E	2,697-4,650	Coleopt. Lepidopt.	Elytron Leg	1 1
20, II	140° /12	185	24° 40'S 176° 30'E 29° 00'S 175° 45'E	4,650	Dipt.	Ceratopogonidae	1

*Alive when caught

† Single entries are at cruising altitude

Table V

Insects Taken in High Speed Trap Between Hawaii and Midway Atoll (Yamamoto)

Date	Wind direction velocity, knots	Plane speed, knots	Lat, North	Long, West	Alt, † meters	Order	Family	No. specimen
1962								
30, IV	240° /20	220	26° 54'	173° 47'	1,860	Dipt.	Acalyptrate leg	1
			27° 40'	175° 57'				
5, V	45° /7		Touchdown Oahu		0-2,480	Dipt.	Nematocera leg	1
26, VI	45° /16		10 min takeoff from Oahu		1,550	Dipt.	Calyptrate abdominal segments	1
26, VI	180° /10	250	24° 02'	165° 40'	3,100	Coleopt.	Ciidae? thorax and abdomen	1
			25° 10'	168° 40'				
27, VI	30° /30	255	22° 50'	162° 00'	5,270	Dipt.	Acalyptrate thorax, leg	1
			22° 10'	160° 20'				
26, VI	95° /14	250	22° 00'	159° 00'	1,000	Dipt.	Acalyptrate tarsal segments	1
			22° 10'	160° 38'				
27, VI	300° /30	260	25° 15'	169° 00'	5,270	Dipt.	Wing fragment	1
			24° 00'	166° 45'				
27, VI	180° /30	245	26° 40'	173° 20'	5,270	Anoplura	Hoplopluridae *Hoploplura pacifica* ♀	1
			26° 10'	171° 10'				
31, VII	110° /20	240	25° 00'	168° 45'	5,270	Homopt.	Exuviae of 1st instar nymph	1
			25° 20'	166° 15'				

† Single entries are at cruising altitude.

Table VI

Insects Taken in High Speed Trap on Antarctic Program (Holzapfel)

Date	Wind direction, velocity, knots	Plane speed, knots	Lat	Long	Alt, † meters	Order	Family	Fragments
1962								
8, X	...	180	Descending		4,572			
			McMurdo Sound		3,810	?	?	Leg
8, X	...	160	Over		3,810	Psocoptera	Liposcelidae	
			McMurdo Sound		1,524			
12, X	220° 30	245	54° 30'S	172° 30'E	5,486	Psocoptera	Liposcelidae	
			46° 05'S	171° 00'E	3,658			
5, XI	290° 25	230	45° 15'S	171° 00'E	3,048	Homoptera	Fulgoridae	Head
			44° 25'S	171° 30'E	1,524			
5, XI	...	...	44° 25'S	171° 30'E	1,524	Diptera	?	Thorax
			On deck—Christchurch, N. Z.		...			
18, XI	360° 40	235	65° 24'S	168° 30'E	3,353	?	?	Leg
			70° 00'S	169° 00'E	4,111			
19, XI	205° 25	236	58° 14'S	168° 08'E	4,877	?	?	Mandible
			50° 40'S	170° 45'E				
21, XI	260° 20	190	77° 20'S	165° 30'E	975	?	?	Claw
			77° 00'S	166° 20'E	1,524			
2, XII	150° 30	202	65° 10'S	169° 40'E	3,353	Hymenoptera	Ichneumonidae	Major fragments
			72° 04'S	171° 25'E				
2, XII	070° 15	190	77° 30'S	165° 50'E	1,524	?	?	Left front wing fragment from Ichneumonidae
			Over McMurdo		915			
14, XII	070° 05	190	McMurdo		884	Diptera?	?	Wing
			77° 30'S	165° 45'E	2,286			
17, XII	254° 45	200	47° 10'S	169° 55'E	3,292	?	?	Mite lost before identified
			45° 30'S	170° 50'E	1,524			
17, XII	220° 10	195	45° 35'S	170° 55'E	1,219	Homoptera	Aphididae	
			43° 55'S	171° 50'E				
22, XII	240° 47	260	24° 10'N	151° 00'W	3,353	Diptera	Chloropidae	Head
			29° 30'N	146° 00'W				
1963								
26, L	280° 40	238	44° 20'S	171° 50'E	2,743	Thysanoptera?	?	Abdomen
			49° 20'S	170° 45'E				
4, II	320° 20	180	Takeoff—Christchurch, N. Z.		...	Homoptera	Aphididae	
			43° 50'S	172° 10'E	610			
14, II	130° 32	246	44° 15'S	171° 20'E				
			49° 54'S	170° 30'E	3,353	Diptera	?	Thorax
5, IV	...	210	Flying airways over New Zealand		3,048	Lepidoptera	?	Leg

† Single entries are at cruising altitude

Table VII

Results of Wind Tunnel Testing of Air Plankton Trap (Lockheed Aircraft Corp.)

Run No.	Configuration	Gate valves	Tunnel vel, knots	Inlet vel, knots	V_A Knots Avg. vel, Sta. A Thru 18 in. dia	Avg. vel, Sta. A Thru 7 in. dia hole	Avg. vel at exit based on meas. static & total pres., knots	Remarks
1A	Trap as designed; 10 micro opening stainless steel screen, with receptacle cup as designed; 10 micron screen lining	Both fully open	136	41.5	2.0	...	11.3	Only low vel run made. Vel boosted for rest of tests.
1B		Both fully open	191	51.2	2.4	...	16.0	Some dirt & dust in cup at end of run.
2	- same -	Aft fully open Fwd. closed at start, opened for 15 sec, then closed	...	...	...	...		Some bugs & simulated bugs put inside inlet & fwd. of container screen at start of run. No bugs in cup. Opened trap at Sta. A; some bugs & dirt here. No pressure data recorded, this run.
3	Same as above but with receptacle cup removed	Both fully open	192	91.1	4.3	...	25.5	Air leaks at fwd. valve assy. Probably present for previous runs. Leaks stopped prior to Run 4.
4A	- same -	Both fully open	193	78.1	3.7	...	25.5	
4B	Installed cup with 2 in. hole in bottom, covered with a flexible 58± mesh screen. (As rec'd.)	Both fully open	194	54.8	2.6	...	19.8	Put several live bugs in cup on installation. Bugs alive & in cup at end of run.
5	557668-1 screen assy. removed. No receptacle cup installed.	Both fully open	193	320	15.0	...	82.3	Base run with screen removed. Container has 3 rings & 2 bulkheads, all with many holes in them.
6	Installed metal diaphragm with concentric 7 in. dia. hole at Sta. A	Both fully open	193	259	(12.2)	80.4 (no screen)	71.8	Run simulating a re-design using larger receptacle area.
7	60-Mesh screen installed on above 7 in. dia. hole	Both fully open	194	245	(11.5)	169	69.2	Screen open area 45% based on assumed 0.0055 in. wire dia.
8	50-Mesh screen installed on 7 in. dia. hole (replacing 60 mesh)	Both fully open	193	242	(11.4)	158	68.4	Screen open area 47% based on assumed 0.0062 in. wire dia.
9A	40-Mesh screen installed on 7 in. dia. hole (replacing 50 mesh)	Both fully open	193	249	(11.7)	150	70.3	Screen open area 52% based on assumed 0.007 in. wire dia.
9B	- same -	Fwd. fully open, aft 1/2 open	195	233	(11.0)	140	80.2	Investigating throttled flow.
9C	- same -	Fwd. fully open, aft 1/4 open (approx.)	195	109	(5.1)	65	51.5	Gate extended down 7.5 in. as measured on vertical dia. of duct.
10	40-Mesh screen still on 7 in. dia. hole. Aft duct assy. (557666-1) removed.	Both fully open	194	291	(13.7)	174	...	To demonstrate that improved flow performance might be achieved if trap exhausts to favorable static pressure field.

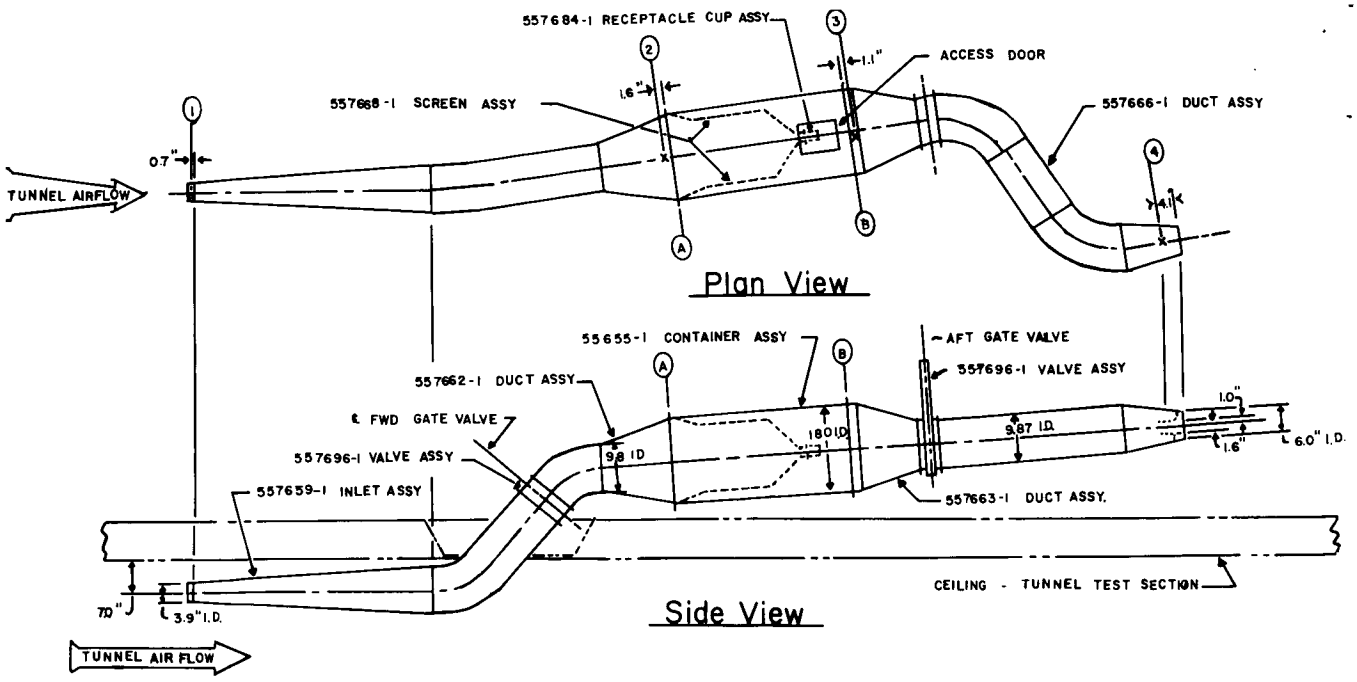

Fig. 5. Plan and side views of air plankton trap showing test setup (Lockheed Aircraft Corp. diagram).

Acknowledgments

For financial support we are sincerely grateful to the U.S. Antarctic Research Program and the National Science Foundation; the National Academy of Sciences, and the Office of Naval Research. Planning was by members of the Bishop Museum with advice from members of the Air Force, Lockheed Aircraft Corp. and from naval aviation architects. Final planning and construction were done by Lockheed Aircraft Corp.

Credit for logistic support and assistance in operating the trap goes to the U.S. Navy, the U.S. Air Force, and the National Science Foundation (Office of Antarctic Programs).

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Discussion

Junge — I found it interesting to hear that you collected nonliving particulates in the air at these altitudes in areas that are remote from continental support. This corresponds to our own experience.

We made a little study recently to find out if there is an upper limit of particles present in normal outside air. It was suggested in earlier publications that there might be an upper limit around 20 μ or so, but, apparently, there is no upper limit. This limit depends only upon statistics; if one collects long enough he gets larger particles. So far we have been able to collect regularly, particles up to 60, 80, even 100 μ radius on a mountain at 2,000, 3,000 meters. Now, these apparently were not from local sources; they must have been carried quite a ways through the atmosphere.

The fact that there is apparently no upper size limit is exceedingly interesting because this may be of importance in spreading particles or microorganisms, etc., around the world.

This is my question: in your table you show some details about the non-living material, about sizes of grains that apparently were quite large. Do you have the approximate corresponding volume of air that was processed during your trips so that you can calculate the concentration in the air?

Holzapfel — To date no applications [of these tables] have been made although we hope for more accurate and hence more valuable data once pending modifications are completed.

We have the calculations of air that entered the trap, but because the screen actually stopped most of the air, I omitted that data from the report.

Junge — Even an approximation would help.

Holzapfel — I don't have our calculations here. The first years, 1960 to 1962, two publications resulted from this trapping. All of the work has been published in the Museum's *Pacific Insects*. Our program started in 1957 on zoogeography and evolution of Pacific insects.

Junge — But you indicated that you also recorded the non-living material.

Holzapfel — Right. This was during the first year of operation before I was even with the Museum. This in in the publication exactly as the table that is here. The technique, etc., I am not totally familiar with. I think many of these were near the ground, naturally, on takeoff. How high they collected these and how valid the data is, I don't know.

As originally planned, the air entering the trap was to pass through the screen mesh and sweep all particles into our collecting cup. The screen actually stopped most of the air, thus preventing contamination from the landing strip from being swept out of the trap between take-off and the first sample. The tremendous turbulence created by the stoppage of this air flow prevented some particles from ever entering the cup; others were collected only after several hours of circulating about in the trap.

Junge — You do not close the traps during take-off and before you collect in view of the contamination near the ground?

Holzapfel — We can very easily close the trap, but normally we found or we thought, that we were sweeping all particles out that may have been collecting when the wind blew at the airport. We kept the trap open to 1,000 meters and then took our first collection. Therefore, the first 1,000 meters certainly would not be a valid indication of what particles were between the ground and that altitude.

Most of our samples were insect fragments. We collected 59 identifiable insect portions, some of which were nearly complete specimens.

Brown — Was the fragmentation produced by the trapping itself, or by something prior to this trapping?

Holzapfel — Both. Some of the small aphids were taken alive and in rather good shape on takeoff, normally up to 1,000 meters. So these certainly would be valid collections. The most impressive was the Ichneumonidae taken near the Antarctic continent. This insect could have been caught in the trap with turbulence and could have stayed there until we made this collection. Since the insect was deeply imbedded, and the entire canister was filled with ice (which indicates that we were flying through clouds) we think the insect probably was collected in the clouds. It was certainly quite dead and fragmented; perhaps, due to the trap's turbulence, but we think due to wind, clouds, and ice action.

Benninghoff — Among particulates we investigated from the snow and ice on the Greenland ice cap, we found about 10 times as many recognizable fragments of arthropods as any other category (spores, mycelia, etc.). Similarly, I understand that in collections from snows high in the alpine region of the Himilayas the same is true. Now, does this result from the fact that at lower latitudes where there is a great deal more vegetation, other particles obscure the remains of arthropods that may be present in equal numbers, or does it result from less degrading biological activity in these high latitudes and high altitudes that allows these arthropod remains to persist without being decomposed?

Holzapfel — Actually, I have no definite comment. The vehicle of collection for our particular program has not given total valid data; we have not theorized any condition like this. We don't have, certainly, these high mountains that you mention in the Pacific; we are trying to find the dispersal of insects between islands and then to study their zoogeography and evolution from this standpoint, so I really can't help you.

Problems of Sampling for Atmospheric Microbes

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Abstract

The atmosphere near the ground typically carries a rich and diverse load of microbes in suspension. We also have some knowledge of microbes in the upper troposphere, but more study, especially on viability over oceans, and at all altitudes in tropical and polar regions is needed. Above the troposphere, with scanty data, we can only speculate on what we might expect, and origins of anything we might find. Technical difficulties of sampling in the upper air are posed by: 1) the large volumes to be tested; 2) aerodynamical problems of removing particles with standard equipment not designed for high intake speeds; 3) problems of retaining viability after capture; 4) limiting contamination during passage through the lower atmosphere and in the laboratory; 5) recognition of contaminants. Future work demands an intensive preliminary testing program against mixed aerosols in a wind tunnel of specially designed apparatus designed after a fundamental study of requirements. This is needed also for sampling atmospheres of other planets. Sampling in space presents quite different problems, but the two techniques would tend to converge near the outer limits of planetary atmospheres.

I make no apology for using the word "microbe" (2 syllables) defined broadly in my dictionary as "An extremely minute living being; whether animal or plant." I prefer this to the word "microorganism" (5 syllables, which the same dictionary defines simply as "a microbe"!).

The atmosphere near the ground commonly bears a diverse flora and fauna of microbes. But we know less about the microbial life in suspension over the oceans, or in the upper part of the troposphere, and almost nothing about the stratosphere and above. We must aim to build up a coherent system of knowledge of microbial life in the atmosphere, and the conditions of the environment that microbial life shares with man and with other large organisms.

As so often happens, knowledge of the microbiology of the atmosphere has had to wait for the development of techniques. Useful information has been gained by examining dust deposited from the atmosphere, particularly on horizontal, sticky slides or culture dishes. But ultimately we need to sample a known volume of air and extract the microbes from it quantitatively. The microbes extracted can then be examined directly under the microscope, or inoculated, either to culture media or to susceptible animals or plants. I shall deal mainly with visual methods.

In this research, selecting the technique appropriate to the object in view is vital. Choice will be influenced by the scope of the inquiry. If we seek a bird's eye view of the whole range of organic particles in the air we must choose techniques that are as *non-selective* as possible. For the large microbes such as pollen grains, cryptogamic spores, and algae, this probably means choosing a visual microscopic method, because almost the only useful property that all these organisms have in common is their visibility under the microscope. This method is less useful for bacteria because they commonly travel attached to dust particles that usually obscure them under the microscope.

On the other hand, when our object is to know more about the occurrence of a limited taxonomic group (even of a single species) or to know more about the organisms sharing some special biochemical activity, then the technique will be made as *selective* as possible: aerodynamical dodges will reject unwanted particle sizes; selective culture media or growth conditions will favor the organisms desired; use of the fluorescent antibody technique may be envisaged. For preliminary reconnaissance in the upper air we should choose non-selective methods.

Atmosphere Near the Ground

Possibilities for the study of individual organisms of importance in health or agriculture are endless. But we still need to map the air spora in its broadest aspect by non-selective methods in many parts of the atmosphere where we have as yet the most fragmentary knowledge. Only in air near the ground in temperate regions has enough been done for some of the main features to emerge.

Progress in this knowledge has been specially advanced by the development by Dr. J. M. Hirst (1952, 8) at Rothamsted Experimental Station of the automatic volumetric suction spore trap. This is a sturdy instrument, making a practical working compromise between many conflicting requirements for continuous air sampling for *visual* examination under the microscope. Some hundreds of Hirst traps are now in use all over the world; one at St. Mary's Hospital, London, England, has been working for 6 years with scarcely a break, except for 2 or 3 minutes each day for servicing.

The Hirst trap has done two main things. It has given precise information about the times in the day, season, and weather, when spores of particular plant pathogens are in the air, and so has greatly improved the timing of sprays and other measures for disease control.

It has also revealed to us, really for the first time, the spore flora of the atmosphere in the ±5-μ range and upward. To me it was tremendously exciting to scan through the first Hirst-trap slides in which almost for the first time the fluctuating air spora was laid out for inspection hour by hour under the oil-immersion lens.

The air spora near the ground is remarkable for its diversity; our catches have provided many puzzles in identification of unknown flying objects. Identification of pollen has already been well developed by botanists, but identification of a fungal spore without the rest of the fungus is only possible in a few species, though luckily a useful number of plant pathogens have characteristic spores.

Some small brightly colored spores on our slides puzzled us for a long time, until gradually it became clear that they were from agarics. In most temperate regions, spores of *Cladosporium* tend to occur in concentrations of many thousands per cubic meter of air in summer *by day*. *At night* basidiospores of mushrooms and toadstools, and especially of the minute leaf-inhabiting "mirror-yeasts" (Sporobolomycetaceae) are extremely abundant (Gregory and Hirst, 1957, 5). In outdoor air at night near Chichester Harbor Dr. T. Sreeramulu and I recorded a million basidiospores of the *Sporobolomyces* type per cubic meter (Gregory and Sreeramulu, 1958, 6). None of these are revealed by the gravity slide method, which is strongly biased toward catching larger particles and is misleading if one wants to infer concentration in air, although the gravity slide is satisfactory if one wants to measure what is deposited on a surface.

Where does the air spora come from? Contrary to the conclusions of many previous workers who assumed that it comes mainly from the soil, I conclude that it comes predominantly from aerial parts of plants, etc., above soil level, though possibly in regions with frequent soil erosion by wind the situation is different. What is in the air at a given time and place is controlled by growth of source, and by meteorological conditions controlling liberation of spores or pollen, and diffusion while travelling on the wind (Gregory, 1961, 4).

To summarize the state of our knowledge from continuous sampling near the ground: we know something about the cryptogamic spores and pollens in the air of temperate regions, their differing diurnal fluctuations in frequency; their diffusion; their occurrence in relation to weather and season. For the tropics, for the colder regions, and over the surface of the ocean we need much more information.

We also need comparable information on concentrations of air-borne bacteria (including actinomycetes) out of doors. In air hygiene, indoor bacteria have been investigated extensively but outdoor bacteria have only occasionally been investigated; a method of continuous sampling in culture for bacteria and fungi is needed urgently — we have scarcely advanced on the diurnal curve for bacteria in the air of Paris given by Miquel (1886, 12, p. 510) (for instance what has happened to city air since the internal combustion engine, with its exhaust, replaced the horse?).

We have no practical sampler at present for continuous trapping in culture, either at ground level or at high altitudes. For spot-sampling we find the Andersen sampler (Andersen, 1958, 1) is excellent — especially for actinomycetes.

Of viruses we have some knowledge only of those assisted through air to animal or plant hosts by insect vectors, and of polyhedral diseases of insects, but of viruses carried on dust, splash, or droplet nuclei we are strikingly ignorant. Yet there is circumstantial evidence for spread outdoors of the viruses of Q-fever, fowl pest, and possibly smallpox.

Middle and Upper Parts of Troposphere

Within the troposphere, but out of reach of the grounded observer, we have some useful information gleaned from aircraft. One cannot be a guest at this University of Minnesota without a glance at history. Even the balloonists contributed information; Blackley (1873, 2), an allergist, used a kite, but the serious study began here in the Twin Cities when Stakman et al. (1923, 18) started to explore the middle part of the troposphere from airplanes; they chased the stem-rust fungus as it moved in spring and early summer from overwintering grounds in the south, up the Mississippi Valley to the prairies. They found viable rust uredospores at least up to 7,000 ft.

North of the 49th parallel Craigie (1945, 3) and his colleagues showed that in most years the rust spore cloud infiltrated in significant numbers to descend upon the Canadian wheat fields; workers farther south showed that in the fall a return migration through the troposphere took place, so honor was satisfied all around. Meanwhile Peturson (1931, 15) made a number of flights over Manitoba and established the vertical concentration profile of rust spores under a variety of conditions. This work has served as a pattern for allied studies in other parts of the world.

Following this, many flights explored the middle troposphere for bacteria, spores of fungi and other cryptogams, and pollen, mainly over land. Some were made over the arctic, such as flights by Lindbergh (in Meier, 1935, 11) and by Polunin (1951, 16) and his co-workers. But study of the atmosphere over land in the tropics has been badly neglected, although it promises to be of the greatest interest.

From Montreal, Pady and Kapica (1955, 13), Pady and Kelly (1954, 14) explored the air routes in two return flights over the north Atlantic for fungi,

bacteria, etc., at up to 9,000 ft. They recorded important differences between air masses of different origins. I don't propose to discuss this aspect now, but one result of these flights is impressive. At 8,000 to 9,000 ft pollen grains seem relatively frequent in contrast with their scarcity in catches just above the water surface made from ships in mid-ocean. The data are all too scanty, but we find a distinct suggestion that in the middle troposphere over the ocean, microbes are more abundant than near the surface of the sea. This finding needs further exploration. Meanwhile an explanation is not hard to find. Dr. Junge provided it in his talk before this group.

The spore cloud in the middle troposphere over the north Atlantic must be made up chiefly of the tails of the distributions of all the point sources on the continent upwind. We can imagine conditions over land and ocean interacting somewhat like this:

1. A spore cloud rising and increasing in concentration as it passes over land, but note that part of this cloud is also deposited on the land, as the land acts as a sink as well as a source.

2. As the cloud reaches the open sea, sources are left behind (only the sink effects remain) and a cleared zone gains height with distance from land, though turbulence continues to bring spores down from overhead.

3. Air below a rain cloud will often be washed almost completely clean.

All these factors could well operate to produce the postulated *greater* microbial concentration at high altitudes over mid-ocean, with smaller concentrations near the surface.

Clearing of the base of the spore cloud has actually been briefly reported by Hirst (1961, 9), using a special suction trap installed in aircraft of the Royal Air Force Meteorological Research Flight. Under somewhat unstable atmospheric conditions, the concentration of most spore types declined logarithmically with height. But the uredospores of *Puccinia graminis* were in greatest concentration at between 3,000 and 5,000 ft. Probably most components of the air spora, such as the mold, *Cladosporium*, originated on the continent of Europe close to the sampling area, in contrast to the cereal rust spores that probably originated from crops in southern Europe, and in respect of which the air mass had been partly cleared from below in passing over non-rusted land areas which were acting as a sink.

Long-distance transport of spores and pollens over the ocean must proceed on a considerable scale. The occurrence of *Notofagus* pollen in peats of the remote island of Tristan da Cunha in the south Atlantic, approximately 3,000 miles from the nearest source in South America, has now been confirmed (Hafsten, 1960, 7).

Upper Atmosphere

If we characterize our knowledge of the middle and upper part of the troposphere as tantalizing and extremely fragmentary, we have to admit that our knowledge of microbial life in suspension in and above the stratosphere, is practically confined to the Explorer II balloon ascent of 1936 (Rogers and Meier, 1936, 17). With renewed interest in the topic, however, we are keenly awaiting reports of new exploration. Meanwhile knowledge can be eked out with speculation.

Excluding minute contributions from emanations from the cabins of jet aircraft, we can imagine three possible sources for microbial life in the stratosphere.

A. ENTERING FROM OUTER SPACE. If any such microbes remain viable we should expect to find them able to tolerate radiation to a degree uncommon among organisms frequenting the lower layers of the atmosphere or else to be carried on cosmic dust particles and micro-meteorites in a position shielded from radiation.

B. AUTONOMOUS INHABITANTS. The possibility of a true aeroplankton, consisting of microbes passing their entire lives in suspension [as suggested by McLean (1935, 10), for the troposphere] is perhaps almost as plausible for the stratosphere, where the risk of removal by rain and snow is negligible. From analogy with microbes surviving well after journeying in the troposphere we should expect these, if they exist at all, to be pigmented and insensitive to radiation.

C. ENTERING FROM TROPOSPHERE. Evidence of some regular mechanisms allowing interchange between troposphere and stratosphere, has been discussed in Dr. Danielsen's and Dr. Junge's papers at this conference.

Any such mechanism could carry air-borne microbes from the troposphere into the stratosphere, even perhaps from the spore-rich *lower* part of the troposphere.

In any case microbial exploration of the upper air should make a definite contribution in its own right to knowledge of air interchange between troposphere and stratosphere.

Sampling Problems of Upper Atmosphere

This new knowledge, however, can be gained only by an extensive sampling program; the conditions imposed at high altitudes raise special technical problems that limit the suitability of any equipment designed for use near ground level. Most of these difficulties arise simultaneously with requirements for sampling in the upper part of the troposphere.

Concentrations in the upper air will probably be very small. Therefore to collect significant quantities of microbes, very large volumes of air will have to be sampled. Conventional apparatus, such as slit samplers, impactors, impingers, bubblers, and filters, are designed to handle a relatively small through-put [flow], of say one cubic meter of air per hour. This probably needs to be increased a hundred- to a thousandfold.

Sampling during a parachute descent probably does not involve any additional aerodynamical difficulties, but this is not true of sampling from moving

aircraft at speeds of 400 mph and upward. Conventional equipment, if it approaches requirements for isokinetic sampling at all, will need re-designing for intake speeds of 100 times those in use at present. Rather than re-design them, it may be preferable to introduce quite novel methods.

If attempts are made to grow trapped microbes in culture, as surely they must be for bacteria, actinomycetes, and yeasts and so on, then the particles must be protected from the air after capture. Viability may be quickly lost if a cell is exposed to a fast moving stream of the same air, in which it could float quite safely in suspension.

The dangers of contamination must be stressed, as they present serious problems at all stages: during preparation, during passage through the lower atmosphere in both directions, and during subsequent handling in the laboratory. Sterilization of equipment and micro-filtration of all air and reagents will be essential (at least during the preliminary stages) until the stratospheric air spora can be demonstrated with certainty at concentrations greater than the level of laboratory contaminants. And in the laboratory unexpected hazards arise from such causes as crawling mites, and molds growing on painted surfaces!

Precautions to be taken against contamination will depend upon the techniques employed. Samples destined for visual examination will not be protected merely by ultraviolet irradiation of the laboratory handling space, because a spore can normally be visually recognizable although killed.

At this point a word must be said on the hypothetical vanishing of spores from the atmosphere. The proteins and carbohydrates comprising spores, pollens and their walls, are exceedingly resistant substances, except to combustion at high temperatures and to the action of specific enzymes — both most unlikely to occur in the troposphere. In fact, except near the ground, the lower atmosphere with its dryness and low temperature may be considered a relatively favorable environment for survival of microbial life. Until further evidence is presented we need not consider the possibility of the air spora losing visibility (as distinct from viability) as likely within the troposphere. This may not be true in the ozone-rich upper stratosphere (or possibly in some chemically polluted ground air), and it would be worth testing the effects of ozone concentrations on spores suspended in air, if indeed this has not already been done.

I must emphasize again that recognition and tracing of contaminants are important precautions that are closely linked with the problem of recognizing trapped objects.

Need for Future Work

With the difficulties that are now apparent, we clearly need a fundamental study of methods for removing microbes quantitatively from large volumes of air at high speeds.

This broad, fundamental study should be treated as a branch of microbiological engineering. Special equipment will have to be designed, and tested in the wind tunnel, for efficiency in trapping mixed aerosols of known composition and concentration. We have passed the stage when all that is necessary is to think of a method and to use it.

One possible approach might be to impact through a forward-facing orifice into a static air cushion. This air cushion could in turn be sampled by more conventional devices. For this, possibly a battery of small cyclone dust samplers, arranged in parallel, could be used to collect the catch into a protective liquid silicone.

Such equipment would be used not only for probing the stratosphere and beyond up to near the limits of our atmosphere, but also for sampling the atmospheres of other planets. Sampling from other planets will pose problems similar in many aspects to those in our own atmosphere.

Quite different techniques will be necessary for detecting microbial life in space. Here aerodynamic problems do not arise; neither do aerodynamics help. The most difficult problems may well be to bring micro-particles travelling at enormous and diverse speeds to the same speed as the sampling device without disruption. The same problem will face us at the confines of our own atmosphere where the two problems converge.

Even more rewarding should be the unpleasant task of sampling lower down in neglected parts of our own atmosphere — unpleasant because it involves flying through the weather. Surely we should push ahead with the adequate exploration of our own atmosphere in time to apply comparisons and techniques when studying the atmosphere of Mars.

The streptomycetes are a most profitable group for study in the lower stratosphere. Their spores range from 0.6, or perhaps even 0.5 μ, up to 1.5 μ in diameter. They are easily liberated in air as single units and they are relatively resistant to desiccation. Unfortunately, we know extraordinarily little about them. If we could compare numbers of spores with those of molds at various heights in the atmosphere, we should go a long way to understanding the movement of organisms in air.

Most present effort seems to be directed toward adapting bacteriological culture methods. This is good, but I want to urge the importance of also using visual methods. It is good that most of the effort is toward the adaptation of bacterial techniques, but cultures are selective. Many interesting and important microbes cannot be grown in culture, at least not by ordinary methods. Examples are the pollens, the rusts, and many other plant pathogens. Cultivation of viable microbes is important, but we must not miss the non-viable and the viable-but-non-cultivable microbes.

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Discussion

Frommhagen — You mentioned the fluorescent antibody technique. To your knowledge, what application has been made of this technique to the study of air samples?

Gregory — None as far as I know. It seems a promising one. We're hoping to use it.

Solomon — Dr. Gregory, on the slides that you showed of Hirst spore trap samples, I noted a grid with which I am not familiar. Could you tell us the quantitative or qualitative significance of the grid superimposed on your collection?

Gregory — The grid is simply a graticule in the ocular lens of the microscope (8). It's used to demarcate areas during counting. It was left in by accident, but it's not a bad idea in fact, because one can remember that a grid square represents a particular volume of air sampled.

Danielsen — There have been many filter samples made with aircraft in the stratosphere to capture radioactive particles; many of these filter papers are still available because they have been saved for isotopic analyses. Could they be utilized? Could they be examined to see if some of the types of materials you're interested in have been captured?

Greene — One of the important things to note is that these experiments have to be designed *before* they go up rather than *after* they go up; if no precautions are taken to preclude contamination both before and after the sampler comes down, and one finds contamination on those filter papers — then what is one going to say? No one would know from where it came. The concentration of this material at ground level is so overwhelming that all you can do is, well, hold another conference to decide what was found.

Gregory — One of the troubles is that filter paper isn't as good optically as a membrane filter. One can make a membrane filter practically transparent, but filter paper is more difficult to use for microscopic examination. This does not matter for radioactive work.

Sampling Microbiological Aerosols in the Lower Atmosphere

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Abstract

Much experience has been obtained in lower atmospheric sampling for viable airborne microorganisms both outdoors and indoors. A large number of sampling devices have been developed for this purpose. For example, 37 different aerosol samplers are described in detail in a recent Public Health Monograph that was published jointly by U. S. Public Health Service and U. S. Army Biological Laboratories. Many of these are not directly adaptable to low temperatures and low pressures that exist in upper atmosphere, but basic principles are applicable in most cases, even though specialized devices will probably have to be developed for any program for sampling in the stratosphere.

As the title of this paper indicates, this presentation will chiefly review some of the basic principles of sampling and a few slides will illustrate some of the common types of samplers used for collecting biological aerosols in the lower atmosphere. The lower atmosphere is being emphasized, because this is where most of the interest has been, where almost all biological samplers have been designed to function, and where we have had the most experience. This information is of interest in the historical development of sampling techniques and while much is applicable to upper atmospheric sampling, this is not always the case. Sampling becomes more difficult at reduced temperatures and pressures and where larger volumes of air must be handled; these difficulties will be touched upon toward the latter part of this presentation.

An investigator in selecting any particular sampling device, should be cognizant of some of the characteristics of the aerosol being sampled and any peculiarities of the organisms in which he may be particularly interested. He must also decide if he wants to know 1) the number of individual viable organisms suspended in a sample of air, 2) the number of particles bearing several viable organisms, 3) the size distribution of the particles, or 4) information relating to all of these factors. He must consider the conditions of the aerosol to be sampled, such as the relative amount of inert material (dust, etc.) present, the environmental factors affecting the aerosol and the probable concentration of organisms. If the purpose of the sampling program is to determine the number of respirable organisms, only those particles of 5μ or less should be investigated because larger particles are usually trapped either in the nasal passage or in the upper respiratory tract by droplets of saliva, and/or by mucous and cilia. These large particles are generally eliminated from the body.

Particulates in a biological aerosol usually vary in size from less than 1 μ in diameter to approximately 50 μ or possibly larger. These particles may be composed of a single unattached organism or may occur in the form of clumps composed of a number of microorganisms. The organism may adhere to a dust particle or may exist as a free floating particle surrounded by a film of dried organic or inorganic material.

Vegetative types of bacterial cells are probably present in the air in a lesser number than bacterial spores, but are more important to health since they include the primary etiological agents of communicable diseases. Many vegetative cells ordinarily will not survive long in air unless the relative humidity and other factors are favorable for survival of the organism or unless the organism is inclosed within some protective cover. Pathogens that resist drying, such as the staphylococcus, streptococcus, and the tubercle bacillus, will survive for relatively long periods, and therefore may be carried considerable distances while still viable, or they may settle on surfaces to be again made airborne as a secondary aerosol during activities such as sweeping.

But interestingly enough, many microorganisms that are still viable in air, having resisted drying and other adverse factors, are killed during collection even though we think the sampling action a gentle one. For example, vegetative organisms that persist dry in air for considerable lengths of time, may die extremely rapidly on dry filters, where they are simply resting on inert fibers with air being drawn past them, rather than remaining suspended in the same air mass.

Methods for sampling airborne bacteria in the lower atmosphere are basically the same as methods used to sample dust and other airborne particulates. Since it is necessary to preserve the viability of the bacteria, while not permitting growth, the existing samplers at times are modified for the recovery of living microorganisms. The basic methods include:

1. Sedimentation
2. Filtration
3. Impaction on solid surfaces
4. Impingement in liquids
5. Centrifugation
6. Electrostatic precipitation
7. Thermal precipitation

For those interested in a review of the basic methods of sampling it is recommended that Chapter II of Public Health Monograph No. 60, "Sampling Microbiological Aerosols," be read (1).

Quite a few different types of aerosol samplers have been used for collecting biological aerosols. Not all of these are adaptable or recommended for routine monitoring; unfortunately many were especially made for certain research studies and are not commercially available. Only a few of these samplers will be discussed, selected mainly to show the various types available. They are not necessarily the most widely used. For a more detailed description of 37 different samplers, reference again is made to Public Health Monograph No. 60.

Various Samplers

Much use has been made of the agar settling plate since it is inexpensive and simple to operate, but the results obtained are qualitative in nature and give little information on the quantitative concentration of airborne bacteria. It is of interest, however, in that it was probably the first aerosol sampler, and unto fairly recent times, it was about the only air sampler in use.

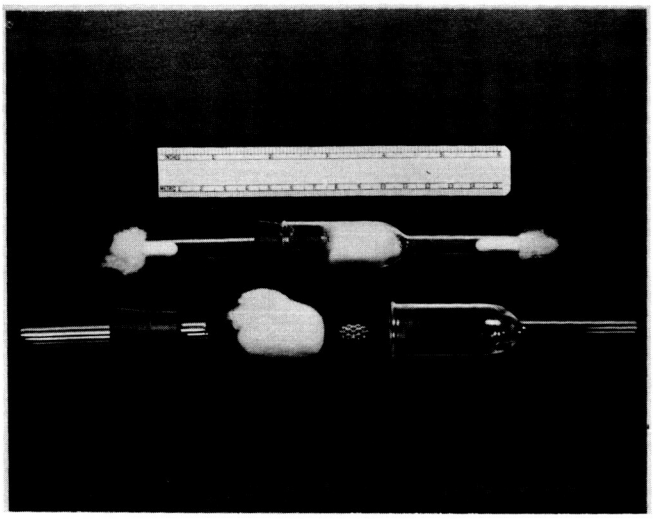
The agar settling plate is simply an ordinary petri dish containing sterile nutrient agar, from which the cover is removed for a time so that the agar surface is exposed to the atmosphere. After this the cover is replaced and the plate incubated. The number of organisms settling depend upon air currents, particle size, and density. This sampler provides an easy and quick method of determining the presence or absence of biological contamination and its quality; however, results can be variable. It has a place in routine sampling, but normally only to indicate the presence or absence of species in question. In addition, use of agar plates at temperatures below 32 F presents numerous problems associated with adherence of organisms to a frozen agar surface, as well as growth inhibition of organisms if a freezing point depressant is incorporated. The agar plate still has its advocates, however, particularly among those who say it best simulates the settling of organisms into an open wound during surgery, for example.

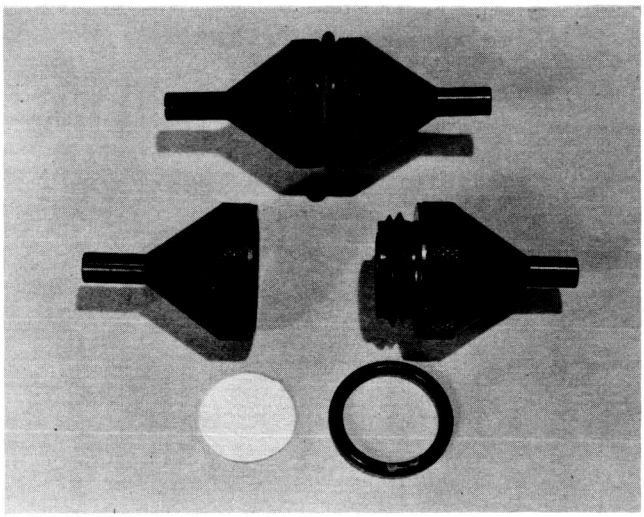
The next simplest type of sampler is that in which air is drawn through some type of inert filtering material, as mentioned earlier. In these samplers, microorganisms are simply filtered or sieved out of an air stream passing through some type of solid filtering media. The usefulness of filter-type samplers depends upon the ability of the microorganisms being collected to resist the drying usually associated with filter collection. Spores and other resistant microbial forms can be readily collected from the air in viable form by a variety of filter media such as cotton (Figs. 1 - 4), glass wool, paper, gel-foam, and molecular filter membranes. Except for the latter type sampler it is necessary to wash the collected microorganisms off the filter or to disintegrate the filter media and then to make plate counts from the wash fluid. The result represents the total number of microorganisms collected rather than the total number of airborne particles containing microorganisms. For those interested in collecting spores, this type of sampler would best fit your needs.

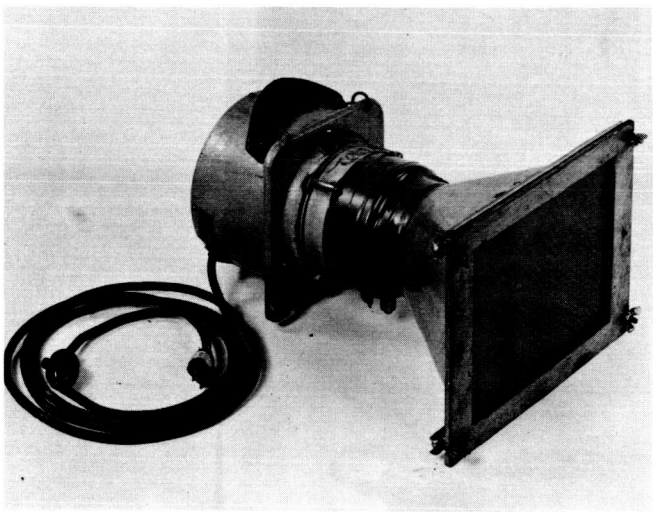
There are other ways besides filtration, however, of transferring aerosols to solid surfaces. With the cascade impactor (Fig. 5), inertial forces are used to collect the aerosol particles. The air passes through a slit a little distance over a solid surface causing the air stream to make an abrupt change in direction. Solid particles not making the turn hit the surface and are held there. This device is of particular interest in that it contains four different stages, in which the slits are progressively narrower so that the air speed and hence the inertial effects are progressively greater causing a separation of aerosol particles on the different stages according to particle size. This instrument was first used to sample inert particles. Only bacterial spores or quite resistant microorganisms will survive this impaction on a hard dry surface, but for those types one not only gets a total count, but the size range into which the aerosol particles fall. Counts are made by washing the organisms off the glass slides into sterile liquid and then plating them by conventional means.

Other less frequently used techniques for transferring biological aerosols onto solid surfaces are those involving electrostatic forces (Fig. 6) or heat gradients (Fig. 7).

Perhaps better suited to the demands of routine use at lower atmosphere are the samplers where aerosols are impacted on a moist nutrient surface. Here the effect on the organism is less drastic than deposition on a dry surface; quite fragile microorganisms can be sampled without serious loss in viability. Impactor type samplers that collect organisms on moist surfaces, however, are limited to the collection of bacteria and fungi but not viruses. Examples of these samplers are the sieve and slit type samplers. The sieve sampler (Fig. 8) consists of an aluminum container that holds a standard petri dish. Air is drawn through small holes and particles are impacted on the agar surface.







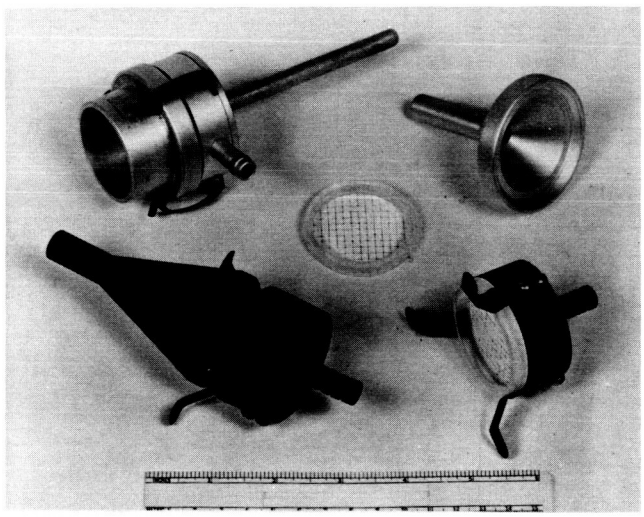
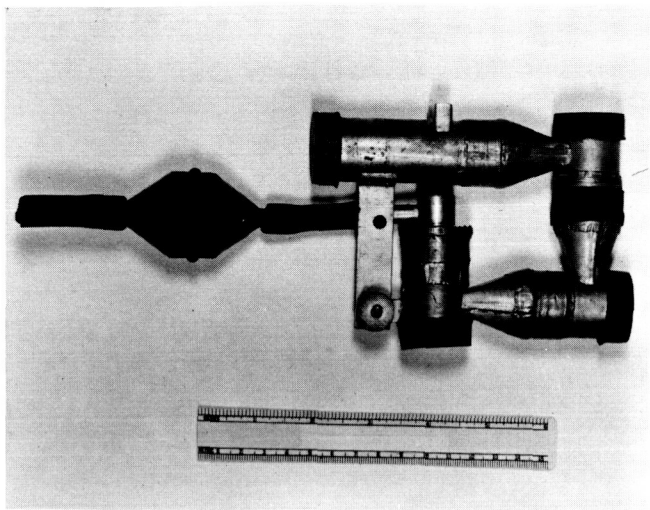


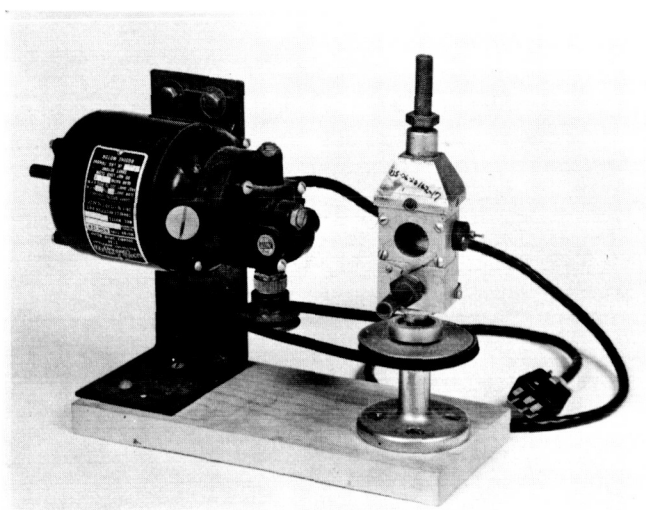
Fig. 1 *(top left)*. Cotton collector.
Fig. 2 *(top right)*. In-line filter paper sampler.
Fig. 3 *(bottom left)*. Modified higher volume air sampler.
Fig. 4 *(bottom right)*. Membrane filter.

The Andersen sampler (Fig. 9) is essentially a cascaded arrangement of six sieve samplers that has holes of decreasing diameter in each succeeding plate. As with the cascade impactor shown earlier, this sampler provides particle size discrimination as well as total bacterial concentration. The Andersen sampler is highly efficient, but the increased information is costly in labor and materials; each time a sample is collected, six agar plates or coated plates must be manipulated.

The Fort Detrick slit sampler (Fig. 10) also deposits microorganisms on a moist agar surface. However it furnishes still additional information concerning the aerosol being sampled, that of time-concentration relationship. Information of this type is desirable when one wants to determine how certain activities, such as bedmaking in a hospital ward, cause a sudden increase in biological air contamination.

The sieve, Andersen, and Ft. Detrick samplers have the advantage of providing qualitative and quantitative information concerning the concentration of airborne organisms in the environment. They are all commercially available. The samples do not require any further laboratory manipulation after collection, since the organisms are impacted directly upon the agar surface; after sufficient incubation, the colonies can be counted directly on the plates. These samplers give, however, not total bacteria counts, but the count of the total number of aerosol particles that contain one or more microorganisms.





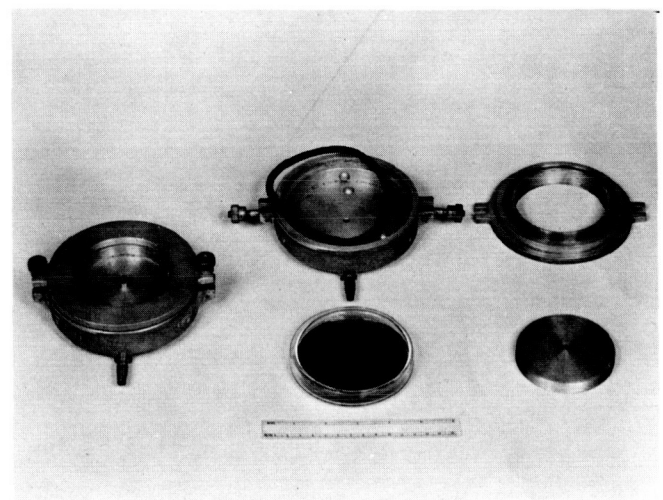


Fig. 5 *(left)*. Four-stage cascade impactor.
Fig. 6 *(right)*. Electrostatic precipitator.
Fig. 7 *(left)*. Thermal precipitator.
Fig. 8 *(right)*. Sieve sampler.

If both bacteria and viruses are of interest to the investigator, a liquid impinger or bubbler can be used. Many types of these exist, but the all glass liquid impinger (AGI) is the most widely used (Fig. 11). Air is drawn through the sampler and organisms contained in the air are impinged in the collection fluid. Normally, samples are collected for a period of approximately 10 minutes after which the liquid in the sampler is assayed by standard biological techniques. This can be used with or without a pre-impinger, that removes the majority of particles larger than 5μ. The liquid impinger requires plating out of the liquid sample; results are best expressed in terms of total organisms, for large particles consisting of several organisms are broken up in this sampler during collection. The data from this source represent not the number of particles found in the air but the number in the disintegrated clumps.

One might expect, therefore, that if a comparison is made between the solid media impaction samplers and liquid impingers, the resulting counts would be higher with the liquid impinger. This could lead to an erroneous conclusion that the liquid impinger indicates a higher concentration of organisms in the air sampled than the other type samplers.

The AGI is only one of a large number of somewhat similar samplers that transfer aerosols into small volumes of liquids; impingers or bubblers similar to the AGI are not illustrated. As a general class, this is the type of sampler against which most others are evaluated since microorganisms are collected without too much physical stress, into buffered liquids where they are in a more favorable environment than when dry in air, and

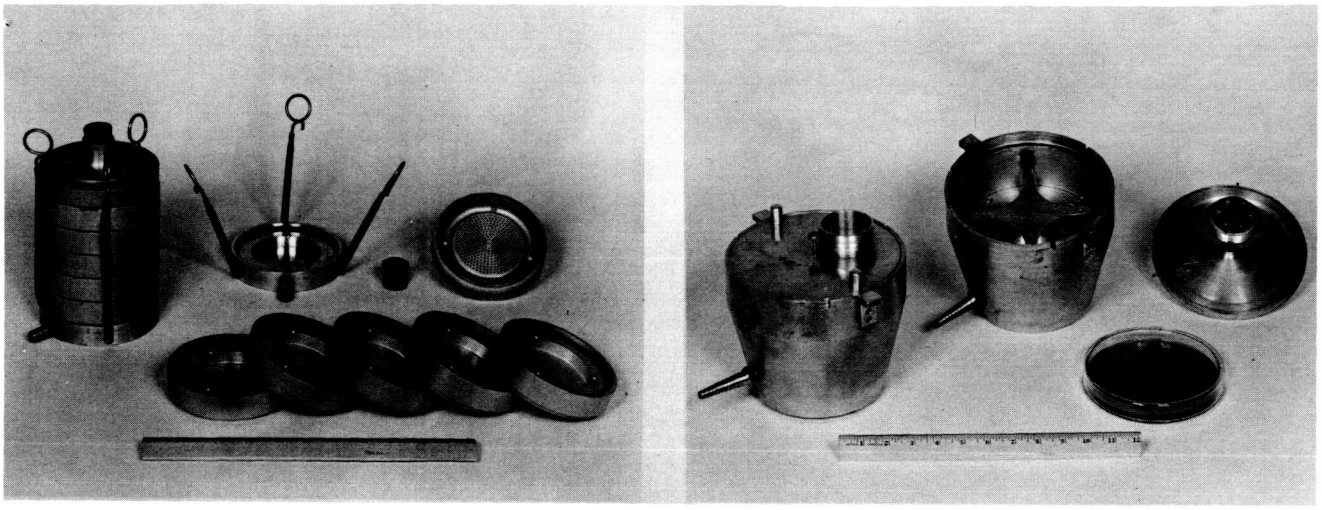


Fig. 9 *(left)*. Andersen sampler.
Fig. 10 *(right)*. Fort Detrick slit sampler.

clumps of organisms are separated into individual cells by the agitation. These samplers almost invariably give the highest number of viable counts.

The last two figures represent samplers that are also concerned with collection into liquids; while neither is too widely used at present, both present another aspect of sampling, one which has application to upper atmospheric sampling. Most of the samplers previously described have been designed for use where microorganisms are expected to be numerous, and precautions often have been necessary to ascertain by short sampling times, sampling rates, or appropriate dilutions that colonies are not too numerous to be counted on plates being examined.

With the Venturi scrubber-type sampler (Fig. 12), fairly large volumes of air (25-850 liters/minute depending upon size of the unit) can be sampled and its aerosol contents are placed in a relatively small volume of liquid that recirculates through the unit.

A quite recent development, a large volume air sampler (Fig. 13), was developed under contract with our laboratories by Litton Industries. Through this sampler 10,000 liters of air a minute are drawn and the particulate content transferred to 10 ml of liquid with high efficiency. Currently glycerol is being used, as a collection fluid, since evaporation is too great at these air flows to use aqueous solutions. Some microorganisms are not too tolerant of glycerol, so other non-volatile, relatively inert solvents that do not affect viability, such as certain silicones, are being investigated. The sampler was designed to collect a large enough number of microorganisms that a sufficient quantity of material would be available for chemical examination, hence preservation of viability was not originally a prime consideration. This is an indication, however, of the direction in which sampler development must occur if effective measurements of viable microorganisms in the stratosphere are to be accomplished. This sampler can handle large volumes of air, operate at low pressures and at low temperatures where samplers that utilize aqueous collection media are inoperable; this sampler also immediately deposits aerosol particles into a small volume of liquid, which can be protected easily against contamination.

Sampling Difficulties

The problems of upper atmospheric sampling are much more complex than are the problems of collection at ground level. This statement is true even when one ignores the auxiliary problems of reaching the stratosphere and having an adequate power source once there. Concentrations of microorganisms in the upper air if present at all are certainly expected to be sparse; thus it is necessary to sample large air volumes. Second, the samples must be brought to ground level without contamination by organisms encountered during descent. With increase in air pressure as the sample is delivered to the lower level, it is imperative that the sample be enclosed in an airtight, leak-proof container to eliminate the risk of biological contamination of organisms certain to be found in the lower atmosphere. This is particularly true if samples are taken on large inert surfaces such as filter paper. If it is impossible to develop an air tight sampler, then an ultra-efficient filter, such as a membrane filter, should be used to serve as a breathing mechanism to permit equalization of pressure.

Another severe problem is that of tremendous temperature changes. This temperature effect is

Fig. 11. All-glass impinger.

twofold. First, it is possible that microorganisms, viable in the cold upper atmosphere, will die off when brought into the warmer temperatures before one has a chance to remove the sample and transfer it into a suitable culture medium. This transfer can be accomplished rapidly, if required, at ground levels. Hours may necessarily elapse, however, before a sample collected above 100,000 ft, for example, even reaches a laboratory.

Another temperature problem is that of sampling at any altitude when temperatures are below freezing. The problem has been insufficiently investigated even at ground level. One reason for this may be that there has been more interest in indoor microbial contamination than in outdoor counts; naturally, building interiors, as in hospitals, barracks, and the like, are almost invariably heated.

Another reason may be that we lack a standard against which to evaluate the efficiency of samplers operating at much below freezing. As mentioned earlier, those samplers, as typified by the AGI, which collect in a liquid medium usually give the highest counts and are used as reference standards. At temperatures above freezing we know that those samplers that collect on dry surfaces are much less efficient, except with the most resistant microbiological forms. These liquid impingers however, become completely inoperative at temperatures much below freezing, since chemical additives to lower freezing points, or heating jackets can make them operative for only a few more degrees below freezing. One suspects that the deleterious effect of collection on a dry surface becomes less severe at temperatures below freezing, but this is difficult to demonstrate experimentally since reference standards are not available.

While not too much is known about microbiological sampling in the stratosphere, several recommendations can be made. One important consideration is that special samplers will have to be developed, just as special purpose samplers have been developed for other requirements. These samplers should be carefully evaluated as to efficiency, before the considerable effort to transport them to the stratosphere for test purposes.

Such evaluation is not necessarily simple. Samplers designed to operate at ground levels have been extensively studied as to their efficiency in evaluating known biological aerosols, in simple aerosol chambers, particularly when compared against one another. Volumes to be sampled are small; no temperature or pressure controls are required, hence such tests can be simple. Chambers simulating the stratosphere are much more complex, elaborate, and expensive, but they do exist. Biological aerosols can be set up in large chambers that operate at reduced temperatures and pressures and the performance of biological samplers can be determined there. Such planning studies should be a prerequisite to developing biological samplers designed to operate in the stratosphere. Much of the experience from ground level sampling is applicable to this problem, but considerable expansion of this technique must be undertaken before we can obtain

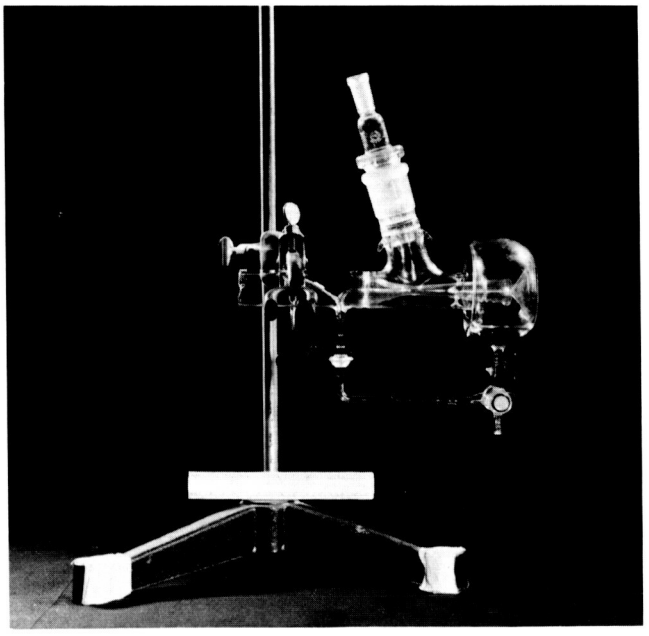


Fig. 12. Venturi scrubber.

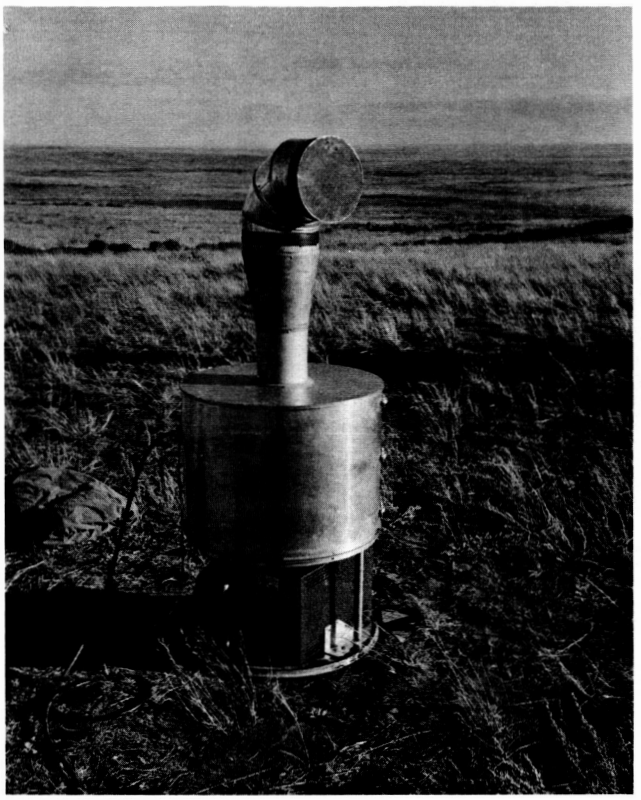


Fig. 13. Large volume air sampler.

stratospheric biological data in which we can have complete confidence.

One problem not considered so far in this paper is what one does with a biological sample, once collected, whether at ground or stratospheric levels. Assessment techniques, media composition and steps undertaken once the sample reaches the laboratory are a related, but different subject, and one which does not depend too greatly upon the altitude at which the sample was taken. No true enumeration of total viable microbiological aerosol count can, under present techniques, be obtained at any level. Aside from the completely different requirements for propagation, and hence enumeration for such different microorganisms as bacteria, fungi, viruses, and rickettsia, one cannot even obtain a true count of any of these broad classes. Consider, for example, bacteria and only the classes of aerobes vs. anaerobes. True aerobes and true anaerobes will not grow on the same media. If one divides a sample, however, and puts half on each type of media, there is always a class of facultative anaerobes that will grow on both, so that the combined counts will contain duplications and be too high.

All that one can say of any biological sample taken either at ground or stratospheric levels, is that it contained certain numbers of microorganisms that grew on various types of defined media, and hence contained at least that minimum of a viable population.

Literature Citation

1. WOLF, H. W., P. SKALIY, L. B. HALL, M. M. HARRIS, H. M. DECKER, L. M. BUCHANAN, & C. M. DAHLGREN. 1959. Public Health Monograph No. 60, U. S. Govt. Prtg. Office, Washington, D. C.

Discussion

Cole – On the large volume air sampler, did you ever look for the larger particles? You were talking about 6 μ on down.

Phillips – I'm sure we had large particles. We weren't particularly interested in them, but at this 5-ft level we should have had some large particles. Some of the glycerine collecting fluid was diluted and examined under the microscope to check on this, following a test with artificial aerosols set up outdoors. The fluid was not only plated out to determine viable count but also examined microscopically to see if we were collecting enough material. Actually, Mr. Lundgren did this work.

Lundgren – The large volume air sampler inlet was at a 5-ft height to correspond to all the other biological samplers which sampled at the 5-ft height. The inlet velocity was about ten feet per second. The screen over the inlet was to keep flies, etc., out. Particles smaller than about 100μ could get through the 125-μ openings in the screen and be collected out by the sampler. These tests were conducted in Canada, where the air was extremely clean. Predominantly all the particles collected were in a size range from 0.1 to 10μ, however, we did find a few 100 μ particles.

Can Spores Survive Space Travel?

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Abstract

Bacterial endospores are more resistant to an unfavorable environment than any other living system known. This discussion deals with those factors that contribute to this resistance. Roles of calcium and dipicolinic acid in contributing heat resistance to the spores and also resistance to desiccation will be discussed. Spores' limits of tolerance to heat will be discussed and also stability of this tolerance during storage.

Resistance of the spore is related to its affinity for water. This brings into focus the question of the role of bound water in spore resistance to desiccation. One needs to ask if the water in spores is bound or if the spores are, in fact, dry in presence of water.

Spores are more resistant than vegetative cells to radiation. Organic compounds containing disulphide bonds such as found in cystine confer a limited protection to biological materials against irradiation. Bacterial spores have been shown to possess macromolecules rich in cystine-like structures. The kinetics of incorporating radioactive S^{35} into cystine-like structures during sporulation indicates that the increase in cystine-rich structures corresponds with the increase of the radioresistance of the cell.

The bacterial endospore is one of the few biological systems that can withstand severe adverse environmental conditions. Spores are able to withstand moderately high temperature, severe and prolonged desiccation, or abnormally high irradiation. In comparison with vegetative cells of the same species, spores exhibit a considerably higher resistance to heat and other unfavorable conditions. It is rather speculative whether or not resistance to heat has developed because of any real advantage which its possession gives to the species, for such high temperatures are rarely met with in nature. This resistance is perhaps present only because of a secondary manifestation of the evolution of a mechanism of survival (1). But one can be certain that this resistance confers on the organism a superior ability to survive under unusual ecological conditions.

The knowledge that the spores are heat-resistant dates back to the experiments of Tyndall and the controversy over spontaneous generation. Tyndall noticed that boiling hay infusions for as long as 5 hours or more did not with certainty render them sterile. Airborne spores caused non-sterility of such preparations. (It is well known that the vegetative cells of the different species vary in their resistance to heat; in general, they can be rendered non-viable by exposure to 80 C for 5 to 15 minutes. The spores, however, are much more resistant to heat than are vegetative cells, although the degree of resistance varies considerably from species to species. A few examples of the survival time of various species of spores at a fixed temperature are presented in Table I. The resistance to 100 C ranges from 1 minute to almost 20 hours.)

Table I

Thermal Death Time of Bacterial Spores

Organism	To kill at 100 C, time, min
Bacillus anthracis	1.7
Bacillus subtilis	15-20
Clostridium caloritolerans	520
Flat sour bacteria	Over 1,030

What is the basis for the thermal resistance of bacterial spores? This question has attracted investigators probably ever since spores were discovered. In spite of intensive inquiry into the nature

of thermostability, we still do not have more than a fragmentary knowledge about the subject. One can visualize the spore as possessing a core containing specialized structures full of normal complements of the constituents of the vegetative cell, but dehydrated and protected by a hydrophobic structural coat. On germination, the hydrophobic barrier is lost, the cell becomes permeable to water, and when suitable concentrations of water are made available the machinery of the cell becomes operative, making the cell ready for growth and division.

An experimental approach to understanding the structure which may be responsible for thermal resistance was initiated by the late Dr. G. M. Hill (10) and continued by Powell of the same laboratory. Dr. Hill found that when he added spores of *Bacillus megaterium* to a nutrient medium they germinated rapidly. Within a few minutes they lost their refractility (used as a criterion) and their heat resistance. Later on, Powell and her collaborators studied germination in order to understand the structure which may be responsible for thermal resistance. On germination the spores of *Bacillus megaterium* excreted dipicolinic acid (DPA), a few peptides, and a large amount of Ca^{++} ions. The residue on evaporation of the "germination exudate" amounted to roughly 30% of the dry weight of the spores (18). Calcium dipicolinate constituted about 50 to 60% of this material. Powell postulated this as a working hypothesis: "the spore is a highly condensed, waterproof structure which has been stabilized by the incorporation of calcium dipicolinate and by the spore coat" (17).

Investigations by several other workers led to the finding that DPA is a normal constituent of bacterial spores (both aerobic and anaerobic). The release of this acid correlates well with the loss in heat resistance; this provides circumstantial evidence for the implication of this compound as one of the essential structural elements for the unique thermostability of bacterial spores. Experiments on the activation of dormant enzyme systems (through heat shock, germination, or mechanical rupture) also show a correlation between release of the DPA and activation of the enzymes (16).

We have demonstrated that the biosynthesis of DPA is complete before the spores exhibit their property of thermostability. In Figure 1 is shown the progress of growth, sporulation, and DPA synthesis in an "active culture" of an anaerobe, *Clostridium roseum*. Note that intracellular structures (which stained like spores) were formed before the synthesis of DPA took place. These structural spore-like forms, however, developed thermal resistance only an hour or more after the maximum synthesis of DPA was complete (8).

Our experiments with an aerobic organism, *Bacillus cereus* strain T, provide further evidence for the fact that DPA plays an essential role in the development of thermal resistance. When we added ethyl oxamate to cultures of *B. cereus* strain T, [grown in a glucose-yeast extract ("G" medium)] the cells progressed normally in their morphological development and formed spore-like bodies which possessed practically no thermal stability [Table II (7)]. These heat-labile spore-like bodies contained only 5% or less of the DPA generally present in heat-stable spores.

Church and Halvorson (3) found a relationship between the DPA content and the rate of thermal inactivation of spores of *B. cereus* strain T. The rate of thermal inactivation indicated a biphasic dependence upon the DPA content of the cells.

As mentioned earlier, a second substance excreted in abundance during germination is calcium in the form of a chelation compound with DPA. Curran's (4) analysis of the elemental inorganic constituents of the vegetative cells and spores of an organism showed that spores contained a high amount of calcium. Vinter (21) observed that the accumulation of calcium correlated with the development of thermal resistance. Experiments with Ca^{45} indicated that the accumulation of Ca^{45} probably coincided with the synthesis of DPA. Our experiments on the

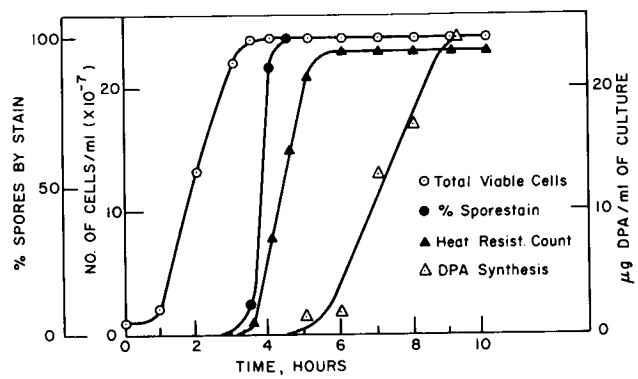

Fig. 1. Progress of growth, sporulation, dipicolinic acid (DPA) synthesis and formation of thermal resistance in *Clostridium roseum*.

incorporation of Ca^{45} in ethyl oxamate-inhibited cultures, however, showed that the calcium accumulation occurred independently of the synthesis of DPA (Table III).

On the other hand, normal DPA synthesis can be obtained when calcium is replaced by strontium; the resulting spores are heat sensitive (20). The thermal resistance of an organism is dependent upon the composition of the sporulation medium. Adding divalent cations such as Ca^{++}, Mg^{++}, and Mn^{++} to the medium resulted in increased thermal stability of the spore (13). Sporulation in low calcium medium produced heat sensitive spores.

The concentration of proteins by a process of dehydration has been postulated as responsible for the resistance of a spore. It has been suggested that the great capacity of the spores for bound water might be causally related to its high Ca^{++} content.

Table II

Thermostability of Cells Obtained in Ethyl Oxamate (10^{-2} molar) Inhibited Cultures of *B. cereus* Strain T

Type of culture	pH	Spore-like bodies octyl alcohol stable cells/ml*	Heat-stable** cells/ml
Spore inoculum		2×10^8	6×10^5
Active culture	5.8↓***	4×10^8	8×10^5
Active culture	5.2↓	7×10^8	1.5×10^5
Active culture	5.8↑	6×10^8	6×10^5
Active culture	7.1↑	1.5×10^9	1.4×10^8
Active culture	7.9↑	8×10^5	2×10^8

*Spore-like bodies remain viable in the presence of octyl alcohol (0.1%) whereas vegetative cells do not.
**Cells are exposed to 80 C for 15 minutes before plating.
***↑↓ Indicates fall or rise of pH in the culture.

This brings up questions of how much water is present in the spore, and in which form it exists. Henry and Friedman (9) reported that spores contained 58% of their wet weight as water while the vegetative cells contained 80% as determined by the loss of weight at 110 C. Using the cryoscopic method, they estimated that a relatively high percentage of water in the spores was in the bound state. These investigations led to several studies about the relationship between moisture and heat resistance (2).

We have examined the activity of spores at different moisture contents as manifested in equilibrium vapor pressure [Fig. 2 (22)]. The spores were dried to constant weight in an electric oven at 110 C while ventilated by a continuous stream of air at atmospheric pressure; they then were allowed to rehydrate by exposure to a moist atmosphere. Samples were taken at intervals during the rehydration cycle; equilibrium vapor pressure and moisture contents were determined by standard techniques. The results showed that vegetative cells were more hygroscopic than spores. In other words, spores exhibited a low affinity for water; the polar groups necessary to attract water were somehow masked. The heat resistance may be a result of "bound protein" rather than "bound water." In other words, the spore produced is dry in the presence of water.

Ross and Billing (19) arrived at similar conclusions by measuring the refractive indices of vegetative cells and spores. They observed the refractive index of spores to be very high comparable to that of dehydrated proteins; they suggested that the spores contained only a little water. Their observations gained further support through the experiments of Murrell and Scott (15), who studied the thermal resistance of bacterial spores at various water activities.

Table III

Incorporation of Ca^{45} into Spores of *B. cereus* Strain T in Presence of Ethyl Oxamate

Time ethyl oxamate added, hrs	Heat-resistant spores, %	Ca^{45} Incorporation*, %	DPA In spores**, %
...	100	100	100
0	0.5	54	3.5
2	8.5	67	4.2
4	5.0	64	4.8

*DPA was estimated by the method of Janssen, Lund, and Anderson (11).
**Ca^{45} incorporation was measured by filtering a known volume of spore suspension through a millipore filter and determining the radioactivity on the filter directly with a gasflow counter.

The high moisture activity of the water in the spores also has a bearing on the resistance of spores to desiccation. The loss of water that takes place during desiccation probably has little effect upon the internal structure of the cells, so they are not injured by drying. This is also true for some vegetative cells, particularly the staphylococci, although, in general, vegetative cells of most species are more sensitive to drying than are spores. It is the general belief of microbiologists that spores in a dry state can survive almost indefinitely, even in air. If a large number of spores is dried and stored in air, a fair portion of the cells will die, but a large portion will remain unchanged for long periods.

Lewis (14) claims that thermal resistance is due to the fact that the calcium dipicolinate squeezes out the water from the cell, so that the proteins are kept dry. He states that anything one does to break this complex (by mechanical rupture or other treatment that will let the proteins absorb water) will cause the cell to lose its heat resistance. He, therefore, maintains that one could even break this resistance by simply applying pressure. He says that if one takes spores on a glass slide and applies pressure with another glass slide, he can break the structure and cause the spores to lose their heat resistance. If this is true then we may need to worry about the impaction method for collecting spores. If spores really hit a solid surface at a high velocity one may well break their structure and lose the heat resistance; the spores may not survive long enough to be grown.

It's common knowledge among investigators working with spores that if one treats spores with any injurious environmental condition such as heat, chemicals, or radiation, the spores will not all die at once. They will die according to a logarithmic die-out curve; this seems to hold true until most of the population has died, when there appear to be a few survivors that live much longer than one predicts by any mathematical formula. These cells also appear to have a long dormancy, so that it is difficult to determine whether or not they are viable.

One experiment was performed by a scientist, who was studying anaerobes. He suspended anaerobes in a growth medium, in glass vials. He sealed the vials by flaming. He then heated them in an oil vat and placed them in an incubator. Most of the cells were killed. He then observed these tubes from time to time to see how long before they became turbid. The last tube began to become turbid after 2 years of incubation.

Let us now briefly consider the resistance of bacterial spores to ultraviolet or ionizing radiations. Interest in this area is rather recent and knowledge of radiation resistance is limited. Spores definitely seem to have a certain amount of protection against ionizing radiations which vegetative cells of the same species do not have (Table IV). Survival-dose curves indicate that lethal dosage is directly proportional to the number of cells irradiated (5).

The physical state in which the cells are irradiated also contributed to the sensitivities of the organism. Koh, Morehouse, and Chandler (12) observed that moist spores of bacilli were more resistant to cathode rays than were dried spores, whereas frozen spores were less resistant than moist spores, but more resistant than dried spores.

Organic compounds containing disulfide bonds such as cysteamine and cystine confer on biological materials a certain protection against irradiation. These compounds are capable of forming mixed disulfides with the SH-groups of the proteins; they offer radiation protection for proteins (6) by acting as "sinks" for the electrons produced by irradiation. This latter observation led to an analysis of cystine-rich components in bacterial spores. The cystine content of spores is about five times higher than that of vegetative cells (22). During the sporulation of *B. cereus*, the cystine content of the cells increased as the young refractile fore-spores developed. The sensitivities of the vegetative cells and the cells with the maximum complement of cystine-rich structure to radiation were determined. More cystine-rich cells survived than vegetative cells. The kinetics of incorporating S^{35}-cystine into the sporulating cells of *B. cereus* correlated well with the increase in radiation resistance of the culture (Fig. 3) (data taken from Vinter, 23).

In conclusion, we can state that spores are equipped with a better mechanism for protection against heat than most living organisms. Furthermore, spores are somewhat more resistant to ultraviolet or other ionizing radiations such as X rays than vegetative cells. It is doubtful, however, that the resistance to ionizing radiation of naked spores is sufficient to protect them during travel in space.

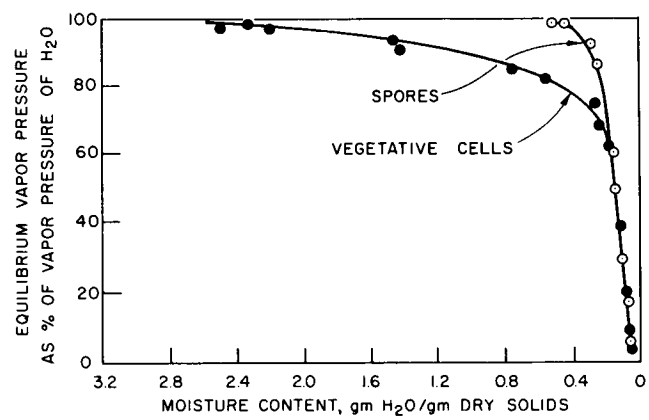

Fig. 2. Relationship between moisture content and equilibrium vapor pressure in spores and vegetative cells of *B. cereus* strain T.

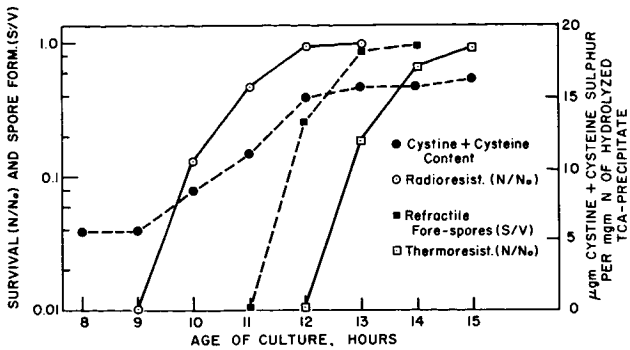

Fig. 3. Changes in resistance to X rays, cystine content, and thermal resistance of sporulating cells of *Bacillus cereus*. N=number viable organisms (at time t) after thermal or radiation treatment. N_o = number viable organisms before treatment. S/V=spores/viable. TCA = trichloroacetic acid.

Thus, if the spores are suspended into space inside of other particulate matter they can survive for long periods of time. These properties indicate that spores may more likely be able to survive space travel than other biological forms.

Table IV

Sensitivities of Vegetative Cells and Spores to Cathode Rays*

Organism	Cell	Concn., cells/ml	Highest dose in megarep showing all survivors	Lowest dose in megarep showing no survivors
B. *mesentericus*	Veg.	2.1×10^4	...	0.3
	Spores	5×10^5	1.3	1.8
Bacillus species E-594	Veg.	1.9×10^5	0.05	0.5
	Spores	5×10^5	1.7	2.0

*Mev, van de Graaf accelerator was used as source of irradiation. (Data taken from 12, Koh, Morehouse, & Chandler).

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Discussion

Bruch — You did not mention anything about the nucleic acids of the spore. There is a recent paper by Kempner (A) of the National Institutes of Health (published in Science on the upper temperature limits). With his methods of analysis Kempner found that the upper limit for growth of vegetative cells is around 73 C. This is close to the melting point for double-stranded DNA. Would you care to make any hypothesis as to what the bacterial spore does with its DNA in order to tolerate the high temperatures that you have shown?

Halvorson — We don't know. Studies on the DNA and the messenger, RNA, and spore and spore-forming cells are just now beginning. A number of us are studying this.

We feel that the DNA content of the spore is the same as that of the vegetative cell. The DNA in spores does melt at about the same temperature as that of the vegetative cells. DNA in the spore is probably different in its orientation or structure from that in the vegetative cell even though the DNA may have the same code. We need to have much more information before we can talk intelligently about this.

Soffen — Can you postulate how the ethyl oxamate might operate in terms of interfering with the dipicolinate?

Halvorson — Unfortunately, I do not have an answer to that. It appears that one of the possible intermediates in the synethesis of DPA has a structure quite similar to ethyl oxamate. We discovered accidentally that it may be an inhibitor (being structurally similar to an intermediate compound) in the synthesis of DPA. Here, again, we need more information. Dr. Srinivasan, my associate, has been able to isolate and identify this compound. We still have to prove that it can be incorporated in the DPA. This, I think, is the answer.

Phillips — I am interested in the moisture content of spores and vegetative cells at different vapor pressures (Fig. 2). We found a different moisture content, depending upon whether we equilibrated a wet spore at various lower degrees of relative humidity, or whether we dried the spores completely and let them rehydrate. You went up to about 40% RH. Was this rehydration or dehydration?

Halvorson — Dehydration.

Phillips — You dried it and then rehydrated?

Halvorson — Yes, that is correct.

Phillips — We found by measuring on the way down that they would be a percent or so higher in most instances. Have you run into this?

Halvorson — We have run into that. I really don't know which one to use.

Phillips — We have also noticed that when a spore is equilibrated at, say, 30% relative humidity (where the cell will have somewhere in the range of 12, 15% moisture usually) it is considerably less resistant than if it has never been thoroughly dried prior to rehydration. We can't figure out why cells which have been completely dehydrated, then subsequently rehydrated to 30% humidity are more resistant than cells also held at 30% humidity, which have never been thoroughly dried.

Srinivasan — I would like to comment on Dr. Bruch's remarks. We have isolated a phage in our laboratory which when used to infect at an early vegetative stage of the bacterium causes lysis just before sporulation. But, when we infect the bacteria with the phage just prior to sporulation after the appearance of the fore-spores, the genome gets into the spore and the spore protects the genome at 80 deg. for about 30 minutes. This particular DNA still carried the genome for the phage multiplication; it can be seen by lysis after germination and outgrowth.

Bruch — I'd like to make a point about the indications that all forms of heat are equivalent. With dry heat I found that many of the spores that have high moist-heat resistance do not have high dry-heat resistance. Would you care to comment on why a spore would be more resistant to dry heat than to moist heat?

Halvorson — I've often been puzzled by the question. When you try to kill a spore with heat, does the heat germinate the spore first and then kill the resulting vegetative cell or does the heat, in fact, kill the original spore? I am inclined to believe that the heat kills by making the mechanism of germination more sensitive to heat than usual. This may have different effects upon the dry spore than upon the wet spore. I don't know how you're going to prove this assumption; I'm inclined to believe that the heat actually induces germination of the spore first, and then kills.

We do know that heating spores does release dipicolinic acid. We know also that there's a rather high correlation between the amount of monodipicolinic acid released and the number of spores killed. I strongly suspect that this acid first destroys the mechanism which protects the cell against heat. Without this protection the cell will be heat sensitive and die.

Church — Would not dipicolinic acid be a reasonable candidate for the sampling trap?

Halvorson — The chemical methods for detecting DPA are not sensitive enough to measure the amount of DPA in a single spore.

Phillips — Instead of about 1 or 2 liters a minute we were collecting 10,000 liters a minute with the last sampler I showed in my talk. We wanted to do chemical work. That is about the ratio between the sensitivities necessary for picking up an individual spore by viable biological techniques (in which there is no trouble at all in determining the presence of one particle 10^{-12} g in weight) and the chemical techniques which are just about many magnitudes less sensitive. You will have to collect much larger volumes than customary. The chemical methods just do not begin to approach the biological ones in sensitivity.

The other factor is that in the stratosphere we're not interested in the number of spore bodies which may be present, but may not be alive. One can still find ATP and dipicolinic acid and a lot of other things after the spore is "killed" (I won't go into what's really a dead or a live organism). When the spore no longer multiplies, those chemicals are still intact for quite a period of time, and still capable of being determined chemically. Their presence does not indicate the presence of life, necessarily; the spores may no longer be capable of generating or multiplying.

Tsuchiya — How much knowledge do we have about the DPA content, the protein content, or the characteristics of the proteins found in thermophiles as compared to mesophiles or psychrophiles? It would seem that the thermophiles are intermediate between the ordinary vegetative cells and the spores.

Halvorson — We know that thermophilic spores contain DPA; thermophilic vegetative cells do not, so the resistance of those vegetative cells to heat is not due to DPA.

Tsuchiya — There is one thing I should like to interject here again. I know this is a difference in point of view. I do not minimize the operational difficulties one bit. We are talking, however, about sending probes to Mars, to the moon, etc. It would seem that the biologist has the obligation of developing the techniques whereby he can detect organic matter or viable material.

Phillips — We need more sensitive methods for picking up small amounts of biological material chemically. That's why we are interested in the development of a large-volume air sampler.

Now, you can, of course, go to the moon, and get large amounts of material which you can analyze chemically to determine whether there is any organic material on the moon as well as if there is any life. The detection of microorganisms which will grow under suitable conditions can be made with a very much smaller amount of material using biological methods than can ever be done using organic chemical methods. Of course, this is with organisms we know. We know the media in which they grow. Maybe we can use only organic analysis for exobiological organisms because we may never find suitable media. But we'll have to try. To determine biological material in a sample size of 10^{-6} g (the weight of a million microorganisms) is easier by standard biological rather than chemical methods.

Literature Citation

A. KEMPNER, E. S. 1963. Science 142: 1318-1319.

Convenor: B. D. Church

An Approach to Study of Microflora in Atmosphere[1]

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Abstract

Living airborne microflora have at least two properties that laboratory microflora do not. First, each airborne cell has survived innumerable changes in environment. Second, each airborne cell maintains its own life in a "dry" and radiation-filled environment where enzymatic or other biochemical reactions, if not absent, are minimal.

Studies of the survival and maintenance of life in conditions like that of the upper atmosphere might be accomplished either by surveying the atmosphere for its living content, or by holding suitable organisms in controlled atmospheric conditions and studying their viability for the purpose of extrapolating to natural conditions. This paper is concerned with the latter approach.

General techniques employed in study of microorganisms in contact with the atmosphere are:

I. Aerobiological techniques, wherein cultures are aerosolized into chambers under controlled conditions, air is sampled, and survival is studied.

II. Freeze-drying techniques, wherein cultures are frozen at various temperatures and then placed into a high vacuum until the moisture content has been reduced to between five percent and some unknown lower limit.

III. Surface drying techniques (desiccation), wherein cultures are painted onto surfaces and then exposed to a variety of atmospheric conditions.

Within these broad approaches, specific methodologies might be utilized to correlate laboratory environment to that of natural conditions. One might vary relative humidity, temperature, and normal gaseous content. One might subject dried cells to irradiation of several types, either artificial or natural. By utilizing a constant test environment, one can explore influence of media, strain, chronological age of culture, or presence of specific chemical additives upon resulting survival of test organism. By maintaining all of these constant, one can study influence of abnormal gaseous constituents on survival. Finally, one can examine the kinetics of action of any one of them, or more generally compare kinetics of one to kinetics of another.

Examples of aerosol studies include influence of short bursts of ultraviolet on bacteria aerosolized from the powdered state. Some cells, while airborne, recovered from what seemed to be lethal treatment. If survivor curves of bacteria, aerosolized after being subjected to changes in temperature in early growth phase, are compared with those grown isothermally, it is apparent that survival in atmosphere is highly dependent upon history of organisms employed.

Freeze-dried cells have been shown to have a gaseous uptake, even at moisture levels below 2%; evidently some metabolic functions continue. The act of rehydration has been studied. Exposure of dried cells to moist conditions before reconstitution was more harmful to cell than just adding liquid, but effect was influenced by temperature. Chronological age of a culture influences way in which cells survive lyophilization and storage. Sampling media influenced the number of cells that grew after storage, but this also varied as a function of chronological age of culture. Temperature of freezing and rate of temperature change influenced subsequent survival in vacuum or in air. Finally, a spontaneous free-radical signal was found in freeze-dried bacteria in contact with air. No signal arose if bacteria died before they were dried, or during the drying step.

The study of bacterial survival undoubtedly involves consideration of population statistics. Each cell seems to have an innate capacity to maintain life in a given environment for a given length of time. This capacity is distributed within population and is time dependent.

[1]This work was sponsored by the Office of Naval Research under a contract with the Regents of the University of California. Reproduction in whole or in part is permitted for any purpose of the United States Government.

Many cells fail to form colonies on specific media when sampled at a given time, but do so if allowed additional time, even though stress condition has not been removed; we call this "recuperation." An enriched medium often failed to support growth of as many colonies from injured cells as minimal medium did.

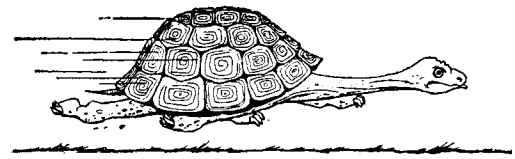
Although current techniques may indicate that a cell was alive at a certain time, they do not indicate if a cell was dead at that time. When survival capacity of cells is examined *in toto*, it is evident that an extremely complex organization within the cell is required for maintenance and survival. In many respects this organization resembles an open-ended, dynamic, self-generating servomechanism. Unless the nature of living cells is held constantly in mind, conclusions drawn from routine studies could lead one into a false sense of simplicity of the problem.

Ever since I read my first science fiction story I have wondered if life exists on other planets. If so, whence did it arise? Assuming that living forms are found in extraterrestrial domains, were they created *in situ* or did they migrate – perhaps from this planet? Can life traverse the vastness of space and spring forth on alien soil? These were exciting thoughts and my imagination soared, but I really did not expect that such questions might be asked seriously in my lifetime. With the event upon us, we must be more pragmatic and we must probe and dissect and finally decide if we can ask such questions meaningfully.

In 1952, as a graduate student about to become a Ph.D., and fully convinced that I had all knowledge there was to know, I stated before a meeting of the local American Rocket Society that any microbiologist would give his right arm to have a handful of moon soil to examine. Now, having lost most of my knowledge somewhere along the way, I would no longer so willingly risk my appendage, for I can see many other questions that need resolution before this simple-minded approach is possible. In the first place, how can one hope to reveal alien life when one does not fully understand life in his own environment; in the second place, if familiar forms are found, how can one be sure that the sampling device was not contaminated by our own atmosphere?

It is necessary to take risks if we are to resolve these questions. For this reason I have included a bit of philosophy (Fig. A). I hope it will perhaps set the pace for what I am going to say.

Throughout this conference we have used words like: viability, survival, stress, recovery, contamination, resistance, injury; alive, dead; kill, adapt. The thoughts we have been invoking imply that we have the ability to detect life and, conversely, death, insofar as single cell forms are concerned, because these ideas are included in the concept of something being sterile and in the concept of living matter surviving stresses of outer space environment. From a more practical viewpoint, these concepts imply that we are aware of what microflora exist in our atmosphere, how they



Behold the tortoise: He maketh no progress unless he sticketh out his neck

Fig. A. Editor's note: While ordinarily such a light note does not belong in the published proceedings of a Conference such as this, the editor has taken exception in this case because it illustrates so readily what the authors are trying to accomplish. (Reprinted by permission of Condé Nast Publications.)

exist, their origin and their capacity to withstand stresses of our own surroundings. Although we actually have little knowledge of these activities at present, they are amenable to study.

We can presume that any viable airborne microorganism has survived innumerable changes in its environment and between changes has also maintained its life under conditions not normally considered ideal; it is a unique cell. Hence we need practice in assaying the survival of microorganisms, and preferably our practice ought to be confined in the beginning to simplified environments. We propose to examine some of the attributes of microorganisms, their life and their survival, and from this examination suggest a general theory that, we hope, will stimulate further questions and lead to possible fruitful areas of new research. Along the way we shall probably not reach complete agreement, but despite this we ought to add something to the knowledge of each of us.

In our laboratory we have been studying the survival of airborne bacteria, principally *Serratia marcescens*, but others such as *Sarcina lutea*, *Pasteurella pestis*, *Escherichia coli*; we have also examined some viruses. We are primarily interested in basic aspects of microbial survival, but some of the techniques and findings should be useful in solving problems normally considered to be developmental.

The principal techniques we employ consist of exposing known cultures of bacteria to the atmosphere and enumerating the living cells as a function of time. Aerobiology is the study of microorganisms dispersed in the form of an aerosol. Organisms are usually grown in shake flasks and can or cannot be separated from the growth medium and suspended in new fluid. The fluid is aerosolized from an atomizer; the aerosol is usually mixed with an air stream of known composition and introduced into holding chambers. These might be of three types: 1) a simple box or barrel having a fan to provide mixing and referred to as a stirred settling chamber (7); 2) a tube or duct wherein the aerosol is conducted in one end and out the other and labelled an aerosol transport apparatus, and 3) finally a rotating cylinder wherein particles fall at one moment toward the axle and at another

moment toward the periphery so that diffusional forces are responsible for most of the physical loss of material; gravitational forces are almost eliminated. Particles about one micron in diameter have been maintained in a rotating drum for up to 2 weeks.

In Figure 1 are shown typical results obtained in a stirred settling chamber. In general it is representative of data obtained with aerosol transport units during shorter intervals of time, and with rotating drums over longer intervals. There is a period of rapid loss of viability followed by a less rapid loss; there is a marked difference between the extent of survival at 53% and at 46% relative humidity (RH). Commonly this effect is delineated by estimating survival in terms of decay constants (an assumed single-hit mechanism) and plotting the constants against relative humidity. When this was done with aerosols dispersed from the dried state (4) (Fig. 2), two areas of minimal survival were noted (15% and 60% RH). The pattern was different from that found with atomized bacteria and the overall replicability was less than satisfactory. We suspected, without proof, that the variability was a result of changes in the cell population, even in the dry state.

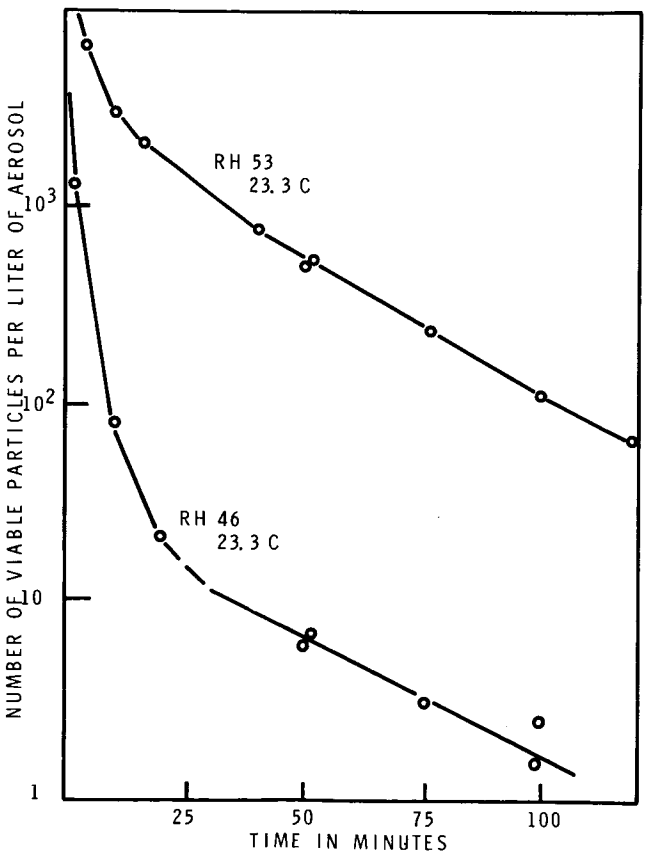


Fig. 1. Survival curves of *S. marcescens* aerosolized into stirred settling chamber. RH = relative humidity.

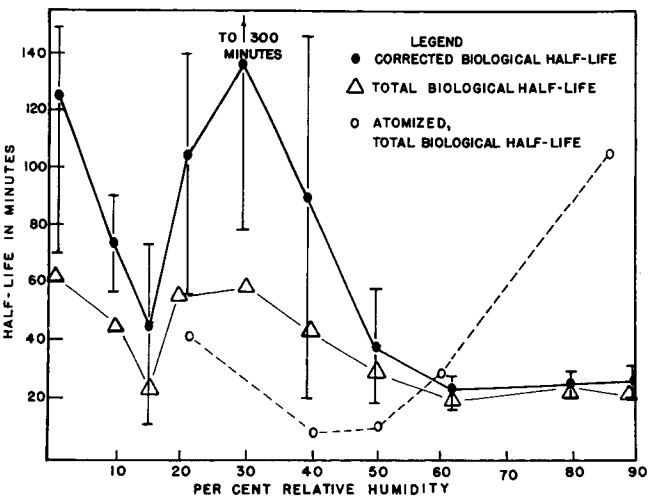


Fig. 2. Survival of powdered aerosols of *S. marcescens* as function of relative humidity, and compared to survival when atomized from fluid media. (Reprinted by permission of Williams & Wilkins Co., J. Bact.)(4).

The difference between dry dispersed *Serratia* and the atomized product aroused our curiosity regarding the effect of ultraviolet irradiation on these pre-dried cells, because we knew that atomized bacteria were quite sensitive. In a stirred settling chamber we exposed aerosols to short bursts of ultraviolet irradiation with the results shown in Figure 3 (5). Approximately 50% died instantly as a result of a 1-minute exposure, but a greater percentage died afterward, over a period of about an hour. After 2 hours, however, the rate of death decreased, sometimes becoming negative – that is, the number of living cells increased. An increase in numbers, usually the result of special manipulation, has been referred to by others as re-activation, but we prefer the term recuperation when the increase occurs spontaneously.

The phenomenon of photoreactivation is not unusual, but when we performed the experiment in the dark, the results were the same. Apparently the classical concept of logarithmic death kinetics cannot always be applied. To test this hypothesis, we grew cultures in shake flasks at 31 C for about 2 hours and then subjected the growing cells to two 1/2-hour temperature changes between 31 C and 16 C to attain synchronous cultures. Although we did not always induce synchrony, we did get survival curves of aerosols from such cultures (Fig. 4). Note that in general the shape of the curves was inverted from that of "standard" cultures and sometimes the number of viable cells increased as noted previously in Figure 3. It was quite evident that these survivor curves could not be explained on the assumption of a single hit hypothesis and that the "state of the culture," in this instance a more homogeneous population than in usual cultures, had some influence on subsequent survival.

Another way of examining the survival of dried bacteria is to lyophilize cultures and expose

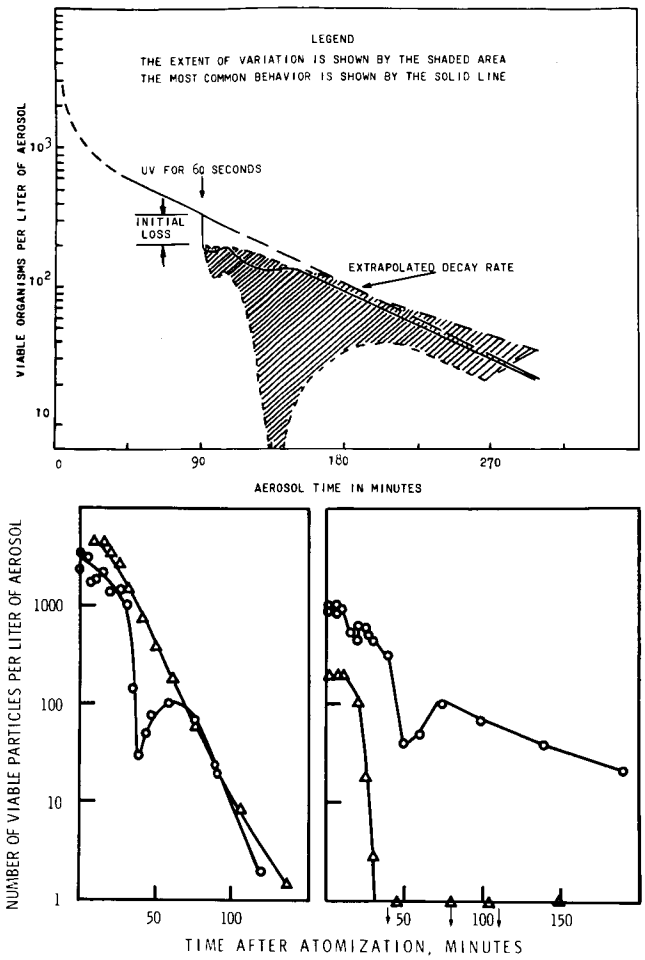


Fig. 3. *(top)*. Survival of 40 powdered aerosols of *S. marcescens* after short periods of irradiation with ultraviolet energy. (Reprinted by permission of Macmillen Journals, Ltd.) (5).

Fig. 4. *(bottom)*. Survival of *S. marcescens* aerosolized into stirred settling chambers after being subjected to alternate temperature changes. Atomizing fluid was a chemically-defined minimal-growth medium.

the dried product to suitable atmospheric conditions. In this way one has a larger quantity of cells to work with and the physical attributes of aerosols (cells per particle, fall-out, sampling problems) are eliminated. The well-known techniques of freeze drying (10) are not difficult to perform, so I will only mention that generally we froze cultures rapidly at -60C and dried them for 24 hours at less than 10 μ pressure to obtain our material. When exposed to ambient conditions, freeze-dried bacteria survived better than aerosolized bacteria, but the pattern of death was about the same. There was an initial rapid loss followed by a slow decline of viable numbers.

Let me digress for a moment to describe some extensive experiments we had been conducting with *Serratia*, including thermal death at moderate temperatures (50-56 C) (6). Briefly, we had found that 1) survival patterns varied rhythmically as a function of age of culture; 2) periods of recuperation occurred at about 6-hour age if the inoculum for the culture was about 10-hours old, but not if it was 24-hours old; 3) diminutive colony forms that did not transmit the diminutive characteristic were induced after short periods of heating but were not found after more prolonged periods, and 4) the number of colonies formed on two different media remained always the same with untreated cells, but varied after cells had been subjected to stress. Physiological activity of cells during stress seemed responsible for some of these unusual findings. Would these same situations obtain with lyophilized cells?

We placed freeze-dried bacteria into microrespirometers, with KOH as an adsorbant for CO_2. We found a slow gaseous uptake (which we presume was oxygen) that varied with moisture content (Fig. 5). It seemed possible that dried bacteria respired, and hence carried out minimal metabolic functions, even at moisture levels of about two percent. If they respired, they were not dormant, and we should expect a constantly changing reaction to the act of reconstitution.

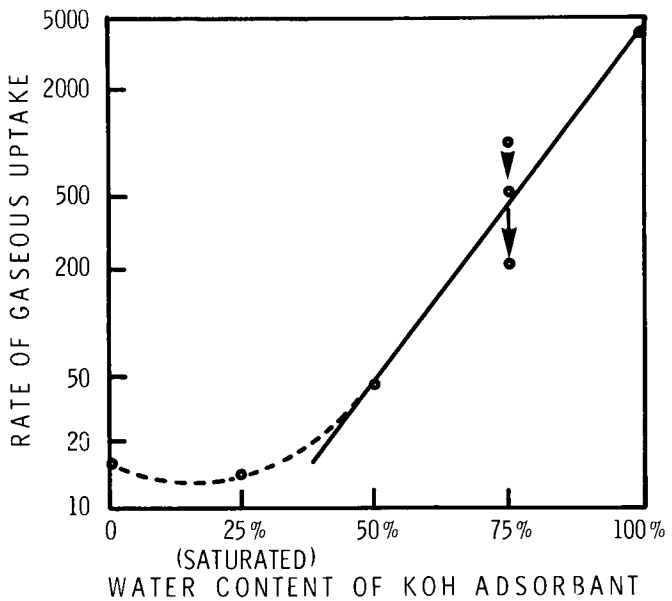


Fig. 5. Gaseous uptake of freeze-dried *S. marcescens* The KOH established moisture equilibrium conditions not determined in this experiment. Rate of uptake, μ liters/day. Rate at 75% moisture was time dependent, starting with upper point, over 6 weeks test period.

In Figure 6 are shown survival patterns after freeze-dried samples from a single growing culture were stored at 21 C. Two freezing temperatures were used, -60 C and -20 C.

Figure 7, a cross-section of part of these data, provides a better example of how survival varied with age. In studying the effect of freezing on the subsequent survival of dried cells one should not ignore the age of the culture.

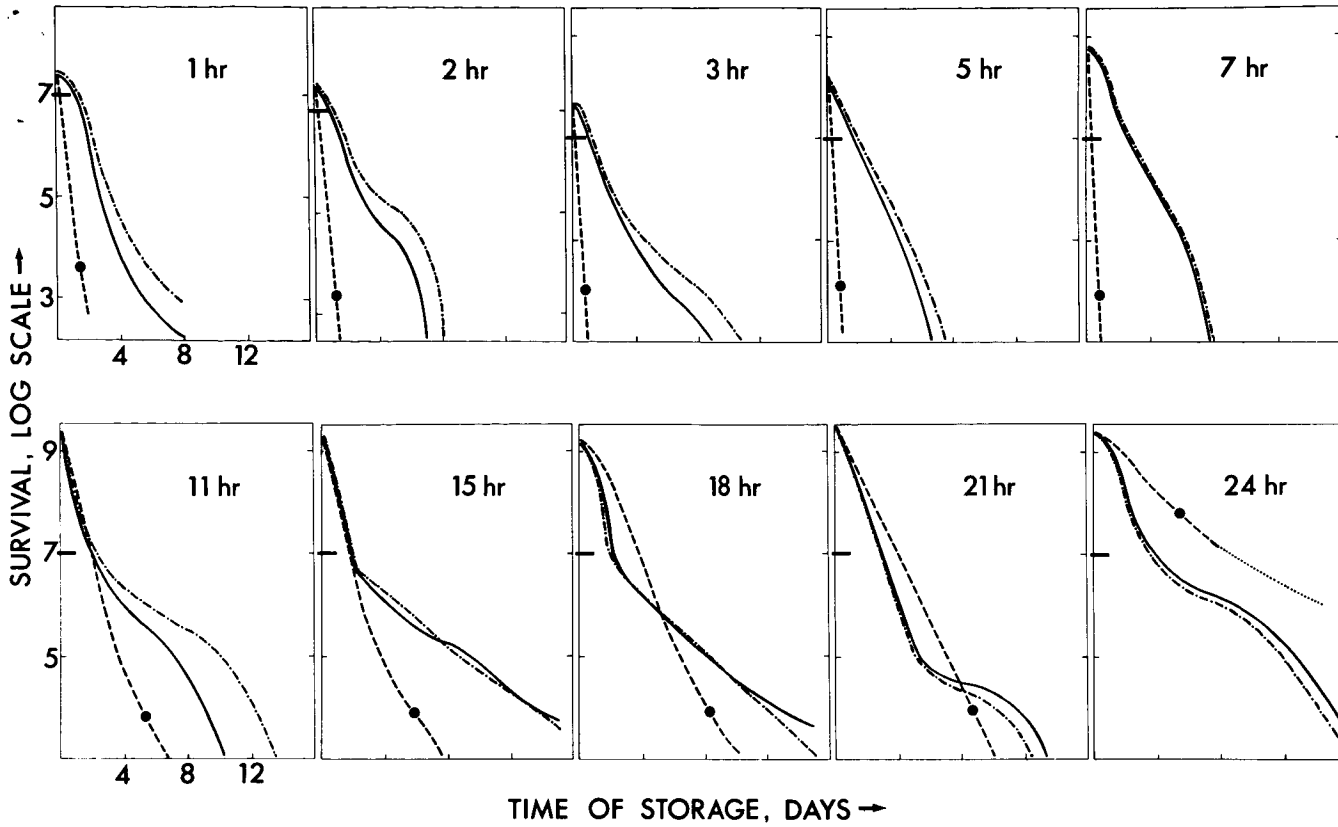


Fig. 6. Survivor patterns of freeze-dried *S. marcescens* stored at 20 C; two freezing temperatures and two plating media. *Solid line*, minimal, chemically defined medium; *broken lines*, the nutritive trypticase soy medium; *dash-dot line*, sample frozen at -20 C; *line with black dot*, sample frozen at -60 C.

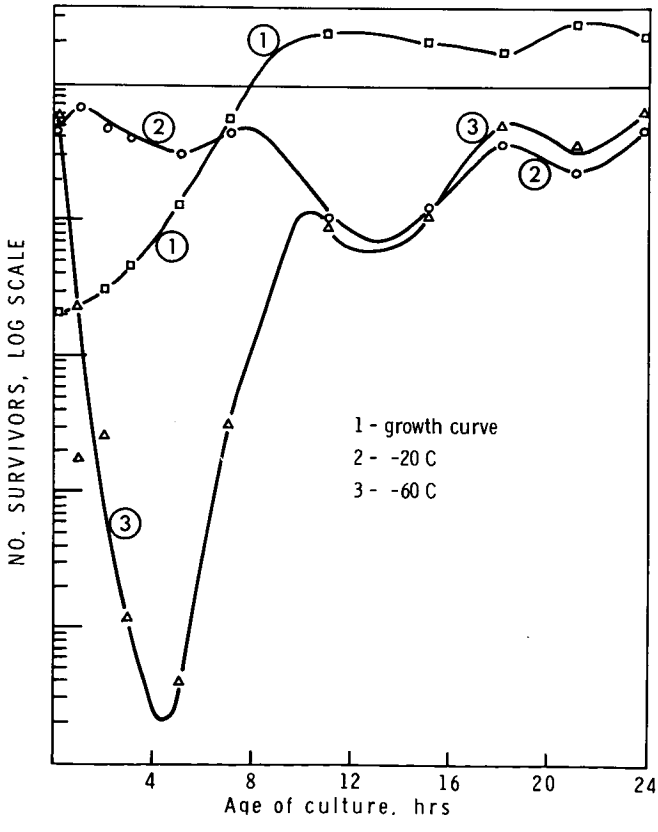


Fig. 7. Storage survival of freeze-dried *S. marcescens* as function age of culture and two freezing temperatures. *Curve 1* is normal curve. *Curve 2* is -20 C, adjusted for differences in initial cell numbers. *Curve 3* is cells frozen at -60 C.

From these same data we plotted the ratio of cells found on one sampling medium to that in the other as a function of age of culture and time of storage (Fig. 8). Sometimes the enriched medium was better and sometimes it was not; there was a marked difference in the whole pattern between cells frozen at the lower and at the higher temperature. Of particular interest, again, is the almost rhythmic way in which these ratios changed. Although these data do not include evidence of recuperation in the lyophilized state, we have other evidence that it can occur in cells grown both on nutrient agar and in flask cultures (Fig. 9).

To add to these complications, the way in which dried *Serratia* were reconstituted influenced their apparent survival. For example, we tested the immediate survival of dry cells in vials immersed in boiling water. Samples were reconstituted by adding water as usual, and by exposing samples either to low or high relative humidity before adding water. In Figure 10 we show that adsorbed water was lethal to some cells, but the extent of lethality varied with length of heating time. This death-by-sorption-of-water (sorbed death) will be mentioned again in relation to aerosols.

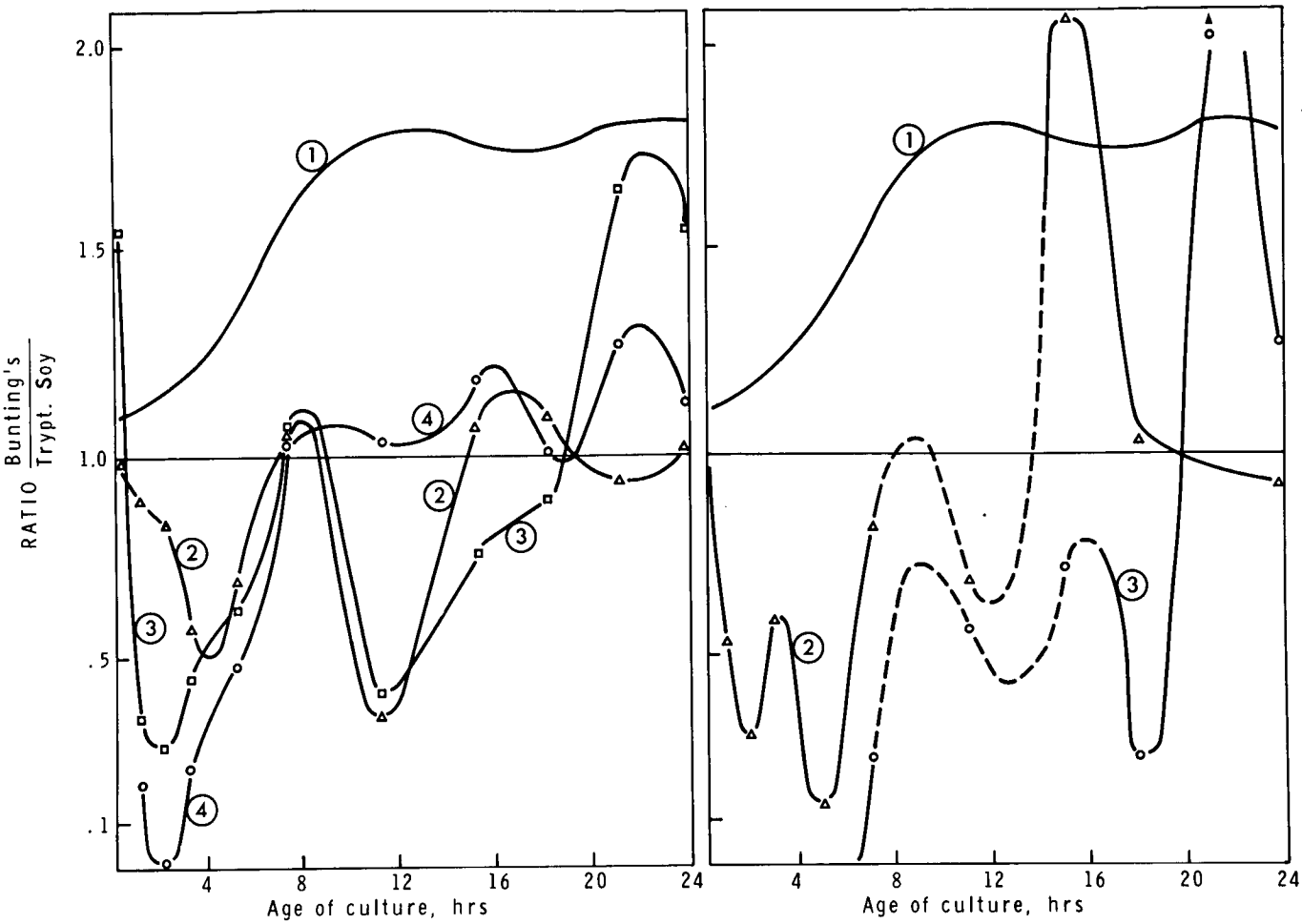


Fig. 8. Change of plating ratio of two sampling media of freeze-dried *S. marcescens*. *Left*, frozen at -20 C, *right*, at -60 C. *Curve 1* normal growth curve. *Curves 2*, 3, and 4 are 2-, 4-, and 8-day storage, respectively.

If cultures were stored at -20C and lyophilized at appropriate daily intervals, then the survivor curves were different, indicating changes had occurred in the frozen state (Fig. 11). In addition, the plating ratio changed, not only as a function of dry storage, but also as a function of pre-storage at -20C. As in thermal death, the history of the cell appeared to be important.

A third technique that might be employed is that of "air drying" microbial suspensions on surfaces and washing them off later to assay viability. There has been a considerable amount of survey work of this nature reported in the literature (14); in general the work bears out our own conclusions that if bacteria were dried slowly, they died rapidly. We finally decided that there must be two principles involved that ought to be separated. The one is the influence of slow drying itself and the other is the influence of surfaces.

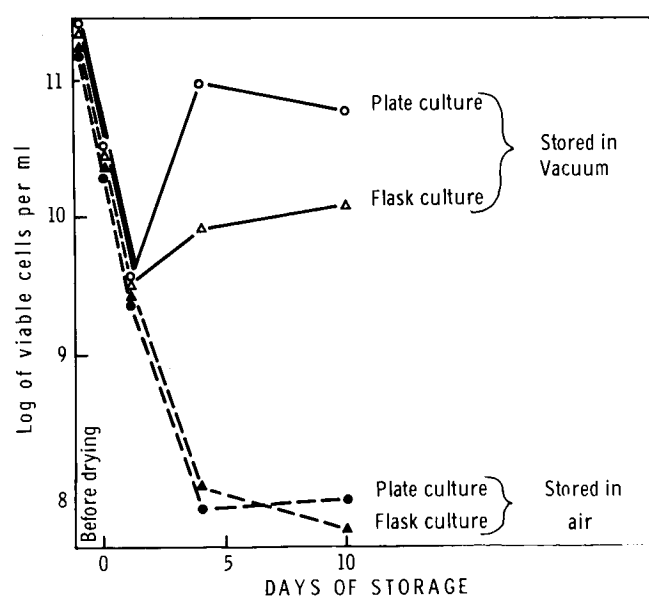


Fig. 9. An instance of recuperation during storage in vacuum of freeze-dried *Escherichia coli* grown by two methods.

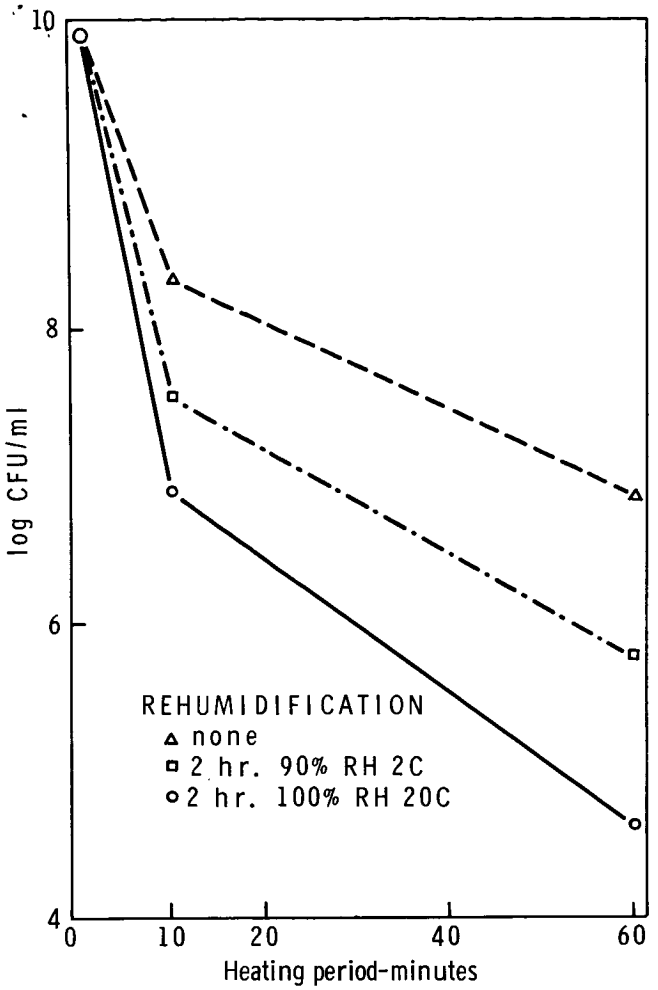


Fig. 10. Influence of rehumidification on apparent survival of freeze-dried *S. marcescens*. Two lower curves illustrate exposure of cells before reconstitution. CFU = colony forming units (see also Fig. 13).

We have not given our full attention to this problem, but we have determined that several species of both bacteria and virus, when spread on stainless steel surfaces and air dried, were unusually sensitive and death was influenced by the relative humidity; in this instance we were testing both drying and storage (N. A. Schlamm, personal communication). If cells were aerosolized, allowed to collect on stainless steel strips, and stored on the strips (one set in a desiccator and one in a plastic box), the survival was not the uniform decay usually found in either aerosols or tests wherein cultures were painted on surfaces (T. R. Wilkinson, personal communication) (Fig. 12). These studies have yielded the most evident periods of recuperation to date.

Perhaps the rate of drying is implicated. A suspension was placed under a sufficient vacuum to remove, but not to freeze the water, as in the snap-freeze technique. The subsequent survival during storage was compared to an identical lyophilized suspension (Fig. 13). Although there were differences in the survival patterns, we can conclude that in general the two populations survived

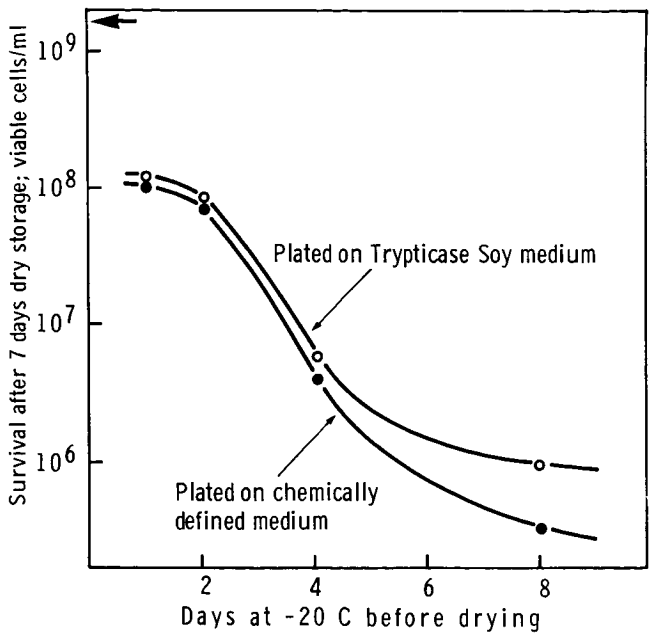
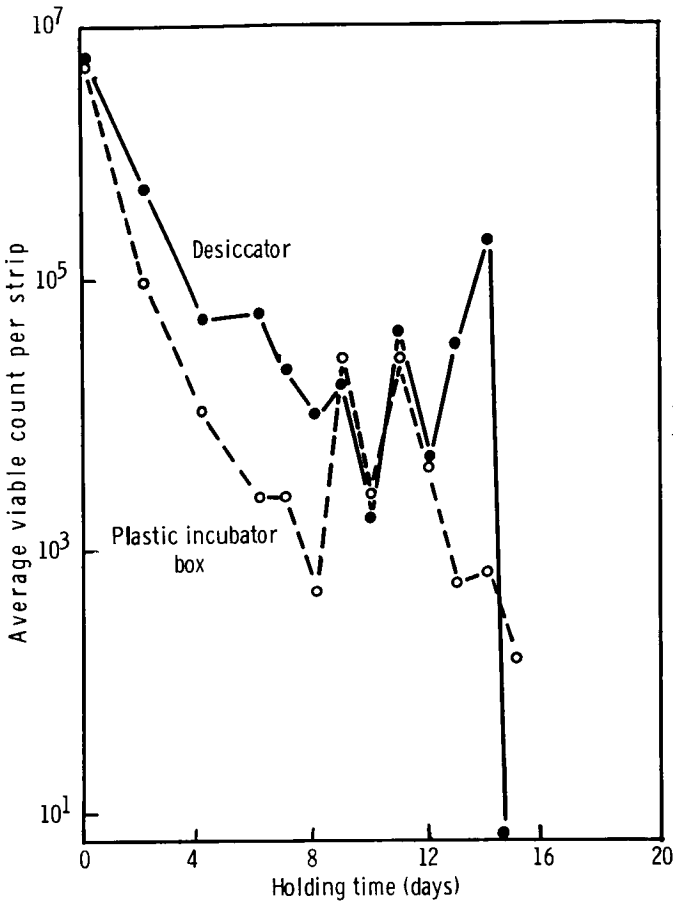


Fig. 11. Change in apparent number of survivors after 7-day dry storage of freeze-dried *S. marcescens* stored at -20 C before drying.

equally well. We know that rapid evaporation cooled the cells to at least 4 C and we presume that the lowered temperature "conditioned" the cells for survival in the dry state, whereas normal evaporative drying kept the cells warm. We shall return to this effect in a moment.

Without further comment, it seems evident that we should direct some attention to a study of changes that occur in cells during stress, or to studies of the influence on survival of environmental changes imposed during stress.

The technique of electron paramagnetic resonance (EPR) (1), sometimes called electron spin resonance (ESR), appears to possess some utility in these studies. Lyophilized bacteria spontaneously produce a free radical if they are stored under conditions that normally lead to death (11). For example, we lyophilized enough cells to permit the packing of several EPR sample tubes under ambient conditions and at the same time a number of vials to be opened for viability assay. All tubes were immediately sealed after packing and stored at different temperatures. A viability assay was performed after 6 days and the results plotted against the increase in EPR signal (Fig. 14). The EPR signal strength appeared to be a direct function of the log of survival. Under certain conditions the extent of life can be measured by electronic means. We do not suggest a causal relationship at this time, but we know, in addition, that if the bacteria are "killed" before drying or are stored in vacuum, then the free radical growth is markedly reduced – sometimes to zero – and that lactose, a substance enhancing survival, reduces the free radical growth.



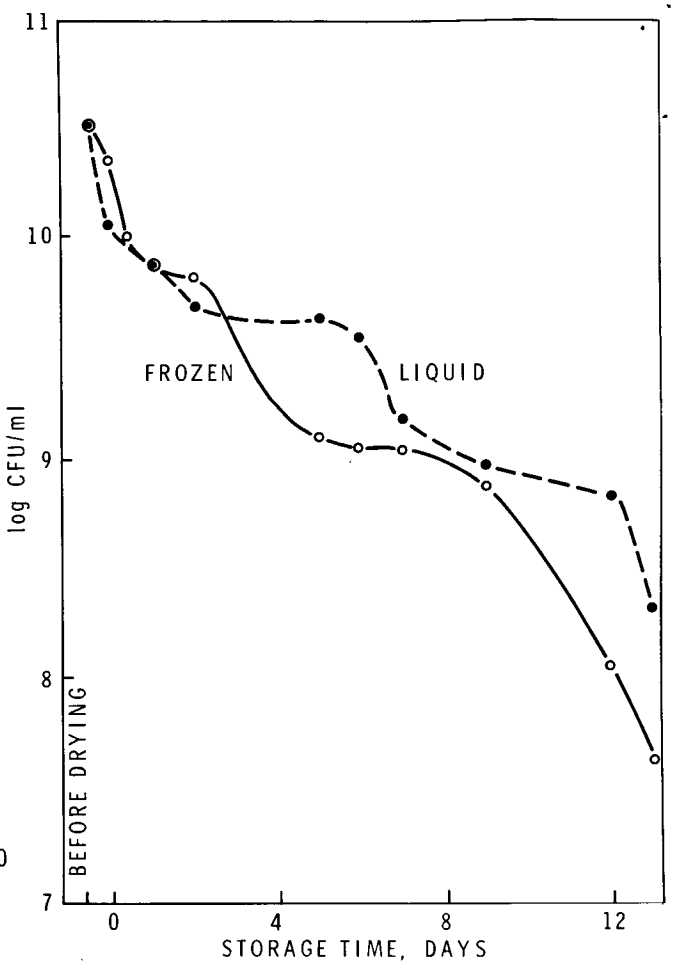


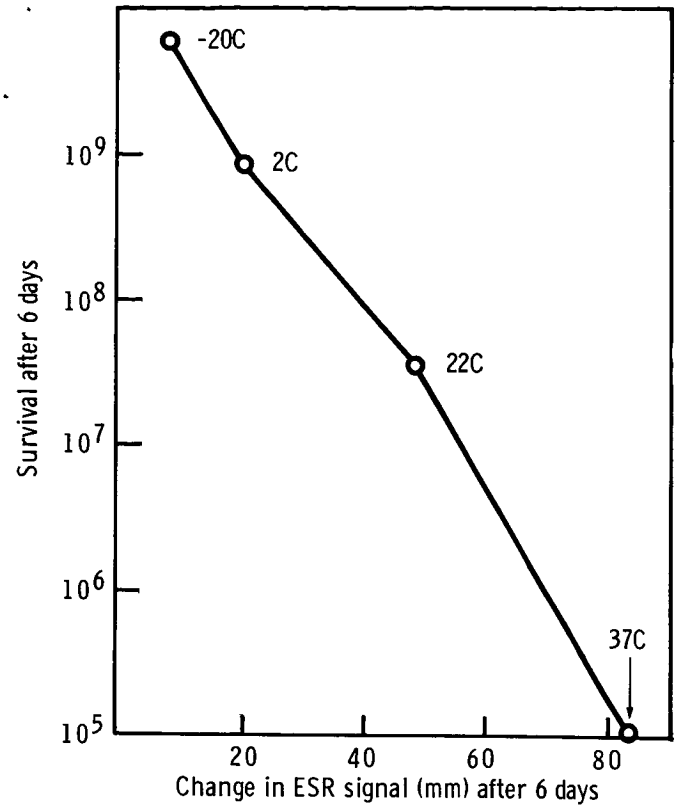
Fig. 12 *(left)*. Survival patterns of *Sarcina lutea* on stainless steel strips at 30 C and 63% relative humidity. All strips exposed to same aerosol (T. R. Wilkinson, personal communication).

Fig. 13 *(right)*. Survival patterns of *S. marcescens* dried with and without freezing. Cells *(upper curve)* cooled to 4 C by vacuum. (CFU, see Fig. 10.)

The radical could be a simple union of oxygen with an organic molecule. But why does this not occur when the cells are dead initially? It is possible that the radical precursor requires oxygen for its development. The radical could be the result of enzymatic activity sparked by oxygen; either the activity caused death or the accumulated radical killed the cells upon reconstitution. Perhaps living cells repress or scavenge free radicals. In light of present knowledge, we can only speculate; nonetheless, EPR can serve as a tool to monitor changes during storage without disturbing the cells.

Finally, we have changed the aerosol environment after the cell has equilibrated with a given condition. We did this by using a dual aerosol transport apparatus. By transporting air at a given velocity through a small duct and then into a larger duct at the same velocity, a 50% dilution of the air stream occurred where the two pipes were joined. At this point a rapid change of humidity could be achieved without a temperature change. We found that a change of relative humidity from 50 to 20% caused no additional death, regardless of growth medium, but a change from 20 to 50% did if cells were grown in an enriched medium and did not if grown in a chemically defined medium (Fig. 15). This sorbed death was almost eliminated if cells were held at 4 C in the atomizer or if antibiotic was added.

Currently we theorize that anything reducing the metabolic activity of the cell before stress is applied tends to enhance survival. This is in marked contrast to Webb's theory (19) that death by drying results only from the breakage of hydrogen bonds in protein structures.



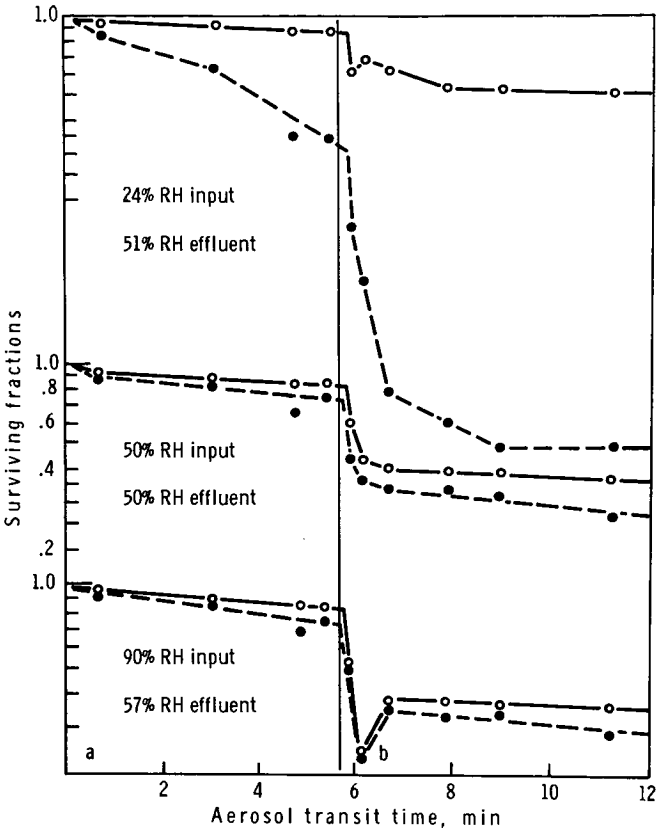


Fig. 14 *(left)*. Correlation of electron paramagnetic resonance (electron spin resonance, see test) signal with survival after 6 days dry storage of *S. marcescens* in air.

Fig. 15 *(right)*. Survival patterns of aerosolized *S. marcescens* as result of rapid change in relative humidity (RH). At just less than 6 minutes, relative humidity was changed from input level to effluent level. Cells grown in an enriched medium.

Conclusions

Considered individually, these data might be analyzed in terms of "textbook" bacteriology, using such explanations as biological variation, mutational frequency, osmotic barriers, cell lysis, clone variation, molecular denaturation, or even in such a mundane term as poor technique. Parsimoniously, the latter is favored, but we have examined this matter thoroughly, and emphatically deny it. Individually, many of our findings are not new (17). Considered collectively, however, together with recent reports in the literature about the semiconductor state of many of the components of living cells (2) (some being of the N type and others the P type, so that the potential exists for putting these together in layers of NPN and producing transistors), these data suggest a more general theory applicable to the whole question of the growth and death of bacteria.

I. Concurrently, we believe that a composite of suggestions by Heinmets (12), Elsasser (8), and Rescigno and Segre (16), and the thermodynamic analogue of Goodwin (9), is the most plausible. A living thing is a unit state of matter wherein electro-physical and electro-chemical feed-back systems perpetuate sustained oscillations of their own substance. Energy is utilized and the system is open-ended and expanding; it has an input and an output. If the system is sufficiently disturbed, an imbalance might occur, leading either to damping of the oscillation or to runaway (i.e., lack of negative feed-back) and the oscillations will eventually cease; the system is dead. A given disturbance might lead to death only if applied at an appropriate moment and only if no other disturbance follows to counteract the initial change.

II. When applied to microorganisms, the theory predicts that a measure of one of the oscillations would be in terms of division time, and we are all familiar with this.

Feedback mechanisms should correct damage to a given component, building new systems if required — we say the cell is adaptable; we find that often cells labelled dead at one time are alive at another; a few grow better on one medium than on another; we say they did not adapt rapidly enough or we say that repressors or derepressors (15) were out of balance.

No two cells can be exactly alike, hence their survival capacity varies, both in terms of the overall components of the cell and the momentary state of the components when stress is applied (13). If the cells are not alike, we should not

expect logarithmic death (18), and we did not find it. Sometimes a large part of the survival data formed a straight line on logarithmic paper, but this is no proof of a monomolecular reaction when we know we are dealing not with molecules but with complex systems. Obviously, if a system is so delicate that it requires the presence of a given component that it cannot make, and if that component is destroyed, then there is a one-to-one relationship between the molecule and the life of the cell. We might, therefore, find strictly logarithmic death, but we cannot claim that all death occurs this way.

III. Our theory predicts that if two living units are close enough to one another, they compose a single system; the input of one is the output of the other. Hence a bacterial culture is usually a collection of systems acting almost as one system. Sometimes small, random changes may be reflected as large changes in a future population; shifts in the distribution of survival capacity within the population should occur as the culture grows, and that is what we found.

IV. Our theory predicts that changes must occur even in the dry state if cells are alive. We found that the plating ratio and the oxygen uptake changed although these changes were not related to survival. This change in EPR signal correlated with survival, but in an inverse manner. Survival in the dried state was related to cell history and recuperation was possible. All our findings point to the idea that the dried cell is dynamic.

Where does this leave us in practical terms? Can we ever understand such a complex system that changes as a result of almost anything we do to measure it? Eisenberg has illustrated a principle of uncertainty in physics and I think we have an analogous principle of uncertainty in biology. If we find a colony on our plating medium as a result of our growth conditions, we can be assured that a living organism was there, but the absence of a colony (and we assume this when we talk about death), does not mean death has occurred — it might just as well mean that the cell could not divide under these conditions at this time. It may not be absurd to consider some cells half-dead.

Can we find a better criterion of life? What we need is additional data, but data from representative species collected for the purpose of creating a "microbial systematics." Scientists might recognize that a microbial culture is a dynamic multiphasic system and determine the influence of age, growth temperature, medium, source of inoculum, plating conditions, timing of operations, and any other suspect variable that should influence the kinetics of survival; they should recognize the need to measure whole cell response, not just characteristics of isolated components. To do this, new techniques will have to be devised. If the data were collected with attention to coordination between groups, then computer technology might be applied and we should finally be able to describe components in terms of systems operating within the living cell, we should know what environmental forces disturb these systems, and eventually we might predict what living forms were to be found in given ecological conditions and how to prove their presence.

In short, if we continue to probe only at isolated problems without attention to the larger and more complex picture (3), then like the blind men and the elephant, without some kind of unified theory and effort, we shall never be able to understand our own life forms, much less those of extraterrestrial origin.

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Nomenclature

EPR	Electron paramagnetic resonance
ESR	Electron spin resonance
CFU	Colony forming units

Discussion

Greene — We've come across some of these same phenomena, especially in the area of thermal inactivation of microbial aerosols. We also found that the results can be varied, almost at will, and that apparent survival is a function not only of thermal exposure, but also depends upon the media, temperature history of incubation, and the culture method. However, this had previously been observed by workers in pasteurization and food canning, who learned about cell injury and the "pampering" of injured, but not yet dead individual cells.

Ultimately we have to return to the pragmatic. The science of bacteriologists is young. The science of bacteriological philosophers is much younger. Before we go off in every direction, however, we should still keep our eyes on the laboratory. There still are some practical problems such as simple experimental errors. I mean the probability of making the wrong observation, for example, day after day.

Dimmick — I can only agree with you in most instances. However, I believe that as biologists we are in a ticklish situation right now because there is a rising group of physicists and physical chemists who are taking a close and critical look at what we've been doing. For a number of years now, biology has been looked down upon by the mathematician and the physicist — largely, I think, because they haven't understood the biologist's problem. When they do, I believe they will explain many of our problems in terms similar to those I have used here.

Now it's time for biology to grow up and to recognize, as you said, that biologists can no longer discard anomalous findings as having been wrong observations made consistently. The only thing new about this is, that if we biologists are not careful, we will find the physicists making inroads into our own bailiwick. The onus is certainly on us to review the whole field of science as much as we can and to try to apply to our own system the scientific knowledge and information borrowed from other disciplines.

I'm not a mathematician, I am not a physicist, and I am certainly not a computer man, but I am fascinated with what can be done with computers, computer theory, and computer technology. The more I look at this area the more I am convinced that we have, at least, an analogous situation with the microorganisms. As a matter of fact, if I'm not mistaken, Weiner (A) developed cybernetics to try to explain some of these biological phenomena. To me, the finding by some people that we *do* have semiconductors in biological material, with the potential of having NPN structures, makes it seem possible for the cell to do things that previously we thought were impossible. We can transfer information in terms of amplification, in terms of electronic oscillation, in terms of feed-back, both positive and negative; the control systems are there. Now if we believe, if we even suspect, that this is the case in single cells, then individuals in populations are going to be slightly different; it is going to be a time-dependent dynamic function. Since we have some evidence that this is the situation, I don't see how we can ignore this fact in our laboratory work, or in the philosophy, if you will, of our research.

Benninghoff — I am a plant ecologist who has dealt with macroscopic plant communities. Knowing relatively little about bacteriology, I have been disturbed in the past by reports on microbial ecology in which environmental relations seem to be resolvable into linear functions or at least into smooth curves, aided in some instances by log normal or log-log plotting, so that the response to environmental stress seems predictable. I am relieved to see that here in some of the responses of bacteria to environmental stress, the history of past environmental stress imposes some directing influence upon the future response to other environmental stresses. Dr. Dimmick also showed that in the contexts of populations of different sizes or mixed species composition the same stress produces different responses. This is precisely the situation we're faced with in dealing with macro plant communities.

Literature Citation

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Microbiological Exploration of Stratosphere[1] Results of Six Experimental Flights

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Abstract

During 1962 and 1963, several balloon-borne probes were launched to determine the existence and identity of viable microorganisms at altitudes between 30,000 and 90,000 ft. Large volume, high efficiency filtration devices were employed to acquire samples ranging from 20,000 to 100,000 ambient ft^3 of stratospheric air, and a variety of controls and precautions were incorporated to preclude extraneous, non-stratospheric contamination.

Despite the best precautions, post impact contamination was still a significant source of microorganisms on the filters. Nevertheless, it was possible to determine that the maximum microbial density above the tropopause was less than 1×10^{-3} organisms/ft^3, and was probably less than $1 \times 10^{-4}/ft^3$. Molds belonging to the genera *Alternaria* and *Cladosporium* were consistently isolated from samplers exposed in the stratosphere.

Historical

During the past 90 years, many attempts have been made to investigate the microbiological distribution in air above ground level (1, 3, 4), yet little reliable information is available about microorganisms in the upper atmosphere (5, 7). Even the extensive investigations at lower altitudes were handicapped by one or more of the following limitations:

A. Lack of efficient sampling devices: attempts to sample microorganisms with such devices as oiled lens-paper filters, sticky slides, and nutrient agar dishes exposed from moving aircraft leave much to be desired. Whereas large particles (>10μ) such as pollen grains and certain fungal spores might be efficiently impacted out of an aerosol, bacterial cells and spores (<5μ) are considerably more difficult to collect by these techniques.

B. Lack of large volume samples and volumetric measuring devices: When sampling the atmosphere where the total microbial population is low ($<10^{-1}/ft^3$), and subsequently returning the sample for analysis to ground levels and laboratory environments where the contamination level is higher by one or more orders of magnitude, a large sample must be taken to increase the signal relative to the noise. Most of the studies reported to date did not sample sufficiently large air volumes to make reliable inferences about microbial concentrations in the atmosphere. Furthermore, many of the previous studies did not adequately measure the air volume sampled, and should be considered essentially qualitative.

C. Inability to sample quantitatively at high altitudes: The ceiling tolerances of manned aircraft built before World War II, and the inadequacies of automatic sampling devices suitable for stratospheric balloon flights limited most aerobiological explorations to the troposphere.

D. Lack of adequate controls and sterility precautions: Many of the previous reports on upper-air sampling may be criticized because of inadequate sterility precautions during assembly, handling, and analysis of the sampling apparatus.

The most significant effort to date in the field of stratospheric microbiology was the National Geographic Society experiment from balloon Explorer II (6). After ascending to 72,000 ft a sterile tube sampler was released to descend by parachute and to sample a profile extending from 69,000 ft to 36,000 ft, where a barometric device

[1]Research performed 1962-1964 under contracts NASr-81 and NASw-648 for National Aeronautics and Space Administration.

closed the inlet ports with cotton stoppers. Sampling was carried out by directing the internal air stream against glycerine-coated walls. The authors assumed that they sampled a column of air 6.6 miles x 3.5 inches or a total volume of approximately 66,000 liters. Upon culturing the recovered sampler, they isolated ten microbial colonies, indicating a density of one organism per 238 ft^3. Despite the obvious criticism that can be leveled against this experiment in retrospect, it is important to note that this work was carried out nearly 30 years ago and that it is a dramatic contribution to biological exploration.

Experimental Approach

In January 1962, we undertook a program of unmanned balloon flights under contract to the National Aeronautics and Space Administration to sample stratospheric air and to ascertain the presence of viable organisms in these samples. In order to avoid many of the problems and limitations that others had encountered in previous explorations, our experimental efforts were guided by the following approaches:

A. The basic sampling process would be air filtration through high efficiency-low pressure drop material from which the entrapped viable particles could be extracted and cultured quantitatively.

B. The mechanical sampling equipment would be adapted from the large volume samplers we had successfully employed in work for the Atomic Energy Commission (2). Attempts would be made to acquire sample volumes in the order of 20,000 to 100,000 ft^3 of ambient air, and to measure the airflow rates and air volumes sampled.

C. Extraneous contamination would be minimized by designing samplers that could be autoclaved; by providing disposable covers and shrouds that would be jettisoned at sampling altitudes; by sampling during a controlled balloon descent, thereby precluding gross fallout from the balloon and equipment; by programming air intake and descent rate to achieve isokinetic sampling; by incorporating a mechanical sealing device to protect the filter from post-impact contamination; and finally by developing aseptic techniques for filter removal and analysis.

Sampling Equipment

The sampling payload finally developed (Fig. 1) consisted of four direct-flow sampling units, mounted vertically on the four corners of a gondola; in the center were nested power packs and the regulating and the recording instruments. The air inlets pointed downward for sampling during descent, and air was exhausted through a high-altitude flowmeter (PR-2) attached to a recording device in the gondola. Air was pulled through the filter by a blower (Torrington No. 704) powered with a d-c aircraft motor (Westinghouse). The skin of the units was of spun aluminum; the frame was of tubular aluminum, and the inlet cone was of sheet aluminum. Each sampler measured 48 inches x 23 inches diameter and weighed approximately 50 lb. The total payload weight of the samplers, gondola, instrumentation, and batteries was approximately 700 lb. It was anticipated that upon impact the inlet cones would collapse and serve as shock absorbers. They were therefore designed to be expendable. The remainder of the unit was designed to be reusable (see schematic drawing, Fig. 2, and photograph, Fig. 3).

Each sampler contained a spring-loaded vertical shaft to which the sealing pans or plugs were attached. This device, designed to minimize post-impact infiltration of extraneous contamination was cocked open during assembly and remained open until after the sampling mission for that given unit was complete. During autoclaving, storage, ascent, and sampling, the shaft was kept in the open position by a squibbed wire cord. At the termination of a sampling sequence, the same switch which de-activated the blower fired the squib to cut the retaining wire, and the shaft was released. A trip lock assembly kept the sealing gates secured after they were closed (see Fig. 2).

Several days before an anticipated flight, the individual sampling units were prepared in the following manner: the units were assembled, and the mechanical sealing devices were tested for reliability. A circular filter of polyurethane foam (80 pore, 1/2 inch x 1 ft^2) was clamped into place. All seals and joints were caulked with Mortite and overlaid with masking tape. The center shaft was fitted with sealing plugs and was secured in the open position. Aluminum-plywood reinforced dust covers were fitted over the inlet cone and the flowmeter exhaust. The latter was shrouded with crimped aluminum foil; the former was shrouded with a weighted nylon sheet which was held in place by a squibbed cinch cord. The completely assembled and shrouded units were then wrapped in surgical wrapping paper and sterilized in hospital autoclaves at 120 C for 1 hour. They were transported to the launch site in their paper wrappers, and stored in this fashion until the arrival of a weather system suitable for launch.

The evening prior to launch, the paper wrappers were removed from the individual sampler units. The units were mounted onto the gondola. The necessary wiring was completed, and the complete payload was placed in a portable vinyl-hypalon chamber, maintained under ethylene-oxide freon pressure (Fig. 4). The payload was kept in this chamber for 12 to 14 hours and was removed to the launch tarmac less than an hour before flight. At this time, the samplers were still being protected by the dust covers and shrouds. Surface samples of the exterior skin had less than one organism per 7 in^2; the filter pad itself was still sterile. The interiors of the samplers were

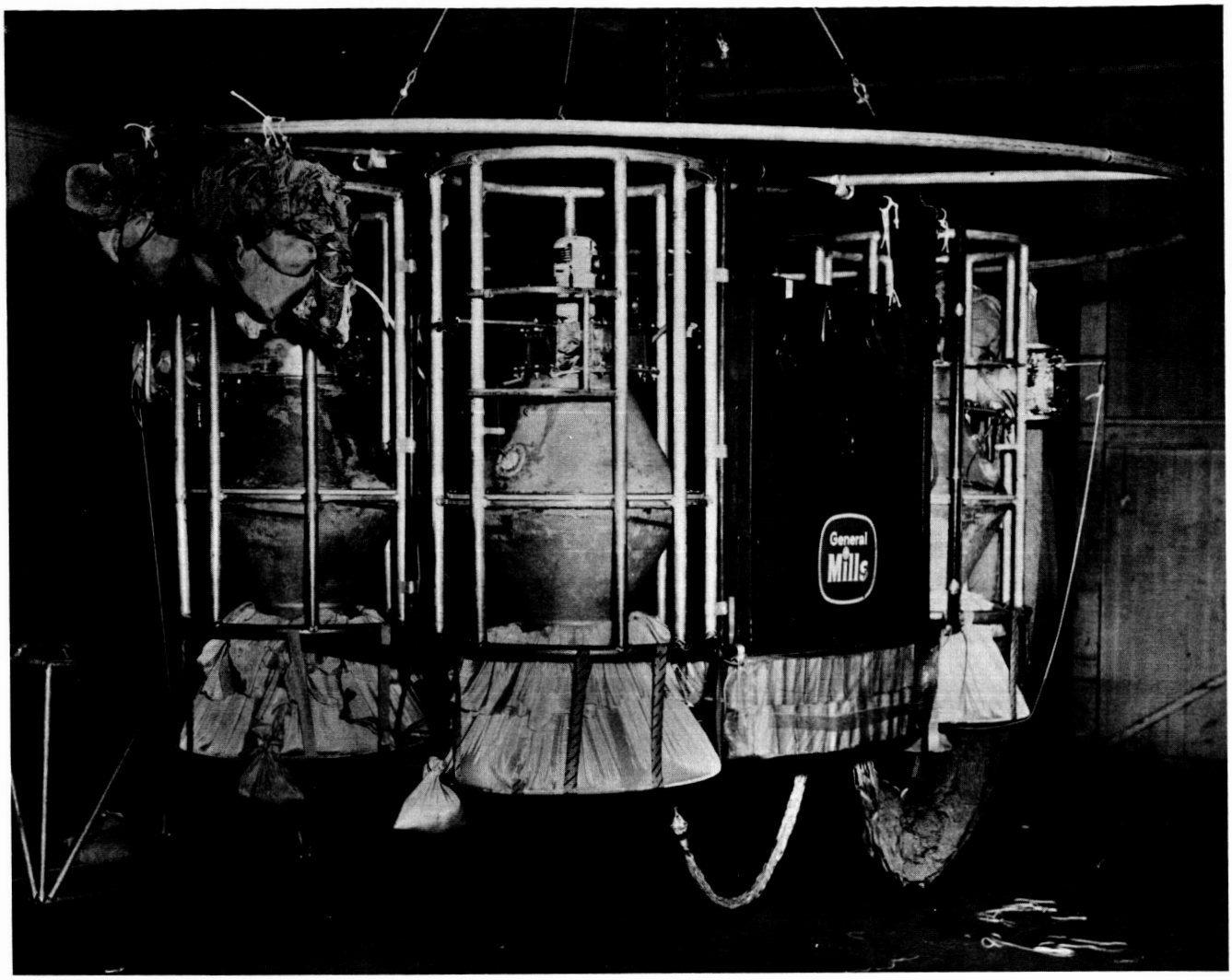
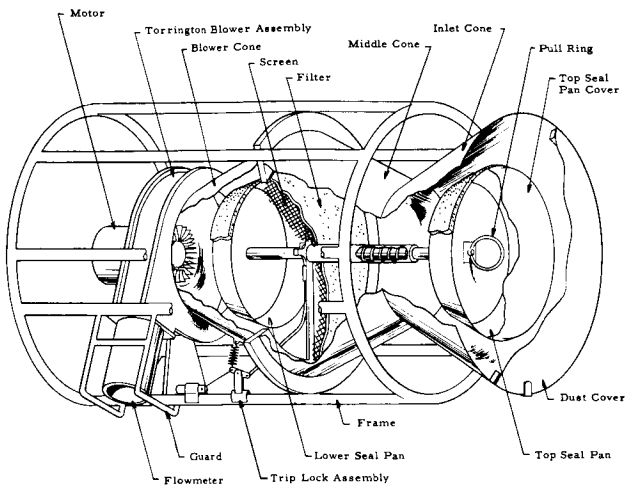


Fig. 1. Assembled payload ready for flight. Note individual sampling units attached to gondola which contains power pack and instrumentation. Inlet cones of the samplers are shrouded with weighted nylon sheets; flowmeters are shrouded with aluminum foil. Shrouds and dust covers underneath are jettisoned at altitude and descend by parachute *(lower right)*. Also note bags of ballast and small chutes *(upper left)* which are dropped by radio command to regulate speed of ascent and descent of payload.

never exposed to contamination between the time of autoclaving and the time of jettisoning the dust covers and shrouds in the stratosphere. After stratospheric sampling, the interiors of the samplers were never exposed between the time the sealing gates locked in the stratosphere, and the time the units were disassembled in a laboratory clean room.

We have tried continuously to improve the sampler (Fig. 5). Most of the changes involved the sealing pans, which proved to be one of the more serious frustrations of this program. Flights 1 and 2 employed metal pans with polyurethane gaskets. Flight 3 used metal pans with silicone rubber gaskets. Flight 4 used a plug made from overlapping sheets of polyurethane. Flights 5 and 6 used cotton plugs held in place with aluminum discs and seated against silicone rubber gasketing in the throat.

Flight 1 used an unshrouded dust cover; the remaining five flights used cinched nylon shrouds described above. During Flight 1 we made no provision for pressure equilibration. In the other five flights we used an absolute fiber-glass filter for this purpose. In Flights 4, 5, and 6, we reached our highest level of internal contamination controls by employing squares of filter foam taped to the interior walls of the sampler. Aerodynamic studies in our altitude chamber showed that these diffusion controls should discriminate between stratospheric organisms (i.e., those in the high velocity airstream) and impact contaminants (i.e., those that leak past the sealing pans).



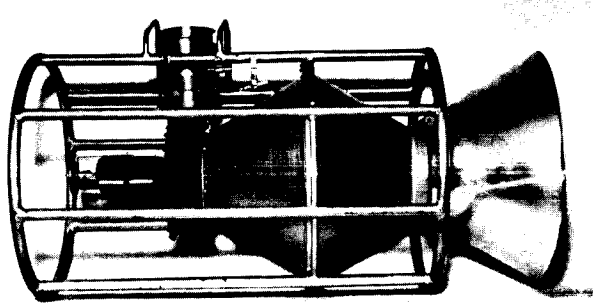


Fig. 2 *(top)*. Schematic drawing of direct flow sampling unit. Sample collected on polyurethane foam supported by wire mesh screen. Note spring-loaded center shaft which closed and locked sealing pans after sampling was completed. Shrouded dust covers (not shown) on inlet cone and flowmeter protected integrity after autoclaving.

Fig. 3 *(bottom)*. Assembled direct flow sampling unit. After attaching dust covers and shrouds individual units were wrapped in paper and autoclaved.

Laboratory Techniques

In Figures 6 and 7 are illustrated the filter recovery and extraction techniques we developed for this program. Essentially the following protocol was followed for analysis of the biological sample in the laboratory:

A. At impact site, sampling units were examined for obvious leaks and malfunctions, detached from the gondola, shrouded in clean polyethylene bags, and returned to the laboratory.

B. Bags were removed, and the exterior surfaces of the units were thoroughly cleaned and disinfected with a phenolic detergent-germicide.

C. Unit was aseptically disassembled in a clean room, and the filter pad was exposed for the first time since completion of sampling in the stratosphere.

D. Filter was dissected into segments: each segment was immediately placed in a sterile polyamide (capran) bag with 100 ml of sterile water; the bags were then heat sealed.

E. The filter was repeatedly and thoroughly extracted with the diluent by manual manipulation.

F. Aliquots of the diluent were removed and filtered through membrane filters (Millipore HA), which were then cultured on a variety of media under different incubation conditions:

1. Tryptone glucose yeast extract agar (DIFCO); 35 C for 48 hours followed by 20 C for 10 days
2. Eugonagar (BBL); 35 C for 48 hours followed by 20 C for 5 days
3. Thioglycollate agar (BBL); 35 C for 48 hours followed by 20 C for 5 days (anaerobically)
4. Mycophil agar (BBL); 20 C for 7 days

G. A laboratory control was obtained by performing steps D, E, and F on a freshly autoclaved sheet of polyurethane foam.

Preliminary experiments, in which segments of polyurethane foam and segments of calcium alginate were uniformly contaminated with both wet and dry microbial aerosols and subsequently extracted, showed that our recovery techniques were satisfactory. Our extraction procedures generally yielded >80% of the total microbial population in the first washing (Table I).

A variety of potentially suitable filter materials was tested for possible use in the sampler: membrane filters, filter paper (IPC), polystyrene paper, Fiberglas, and polyurethane foam. After considering filtration efficiency; chemical and biological inertness; low pressure drop; stability in simulated stratospheric environments; ease of sterilization; ease of manipulation, and ease of recovery of organisms from filter matrix, we chose polyurethane foam as the filter material. After a flight, the filter could easily be dissected out of the apparatus and the organisms adhering thereto quantitatively extracted. In Table II is shown the efficiency of polyurethane foam against microbial aerosols (ca. 1 μ diam.) artificially generated in an altitude-simulation chamber.

We performed a series of trials to determine if filtration might be inimical to viability, and if vegetative cells deposited on a polyurethane filter would be killed by blasting air across them at high velocities (Table III). Lyophilized *Serratia marcescens* exposed to airflows of 1,000 fpm

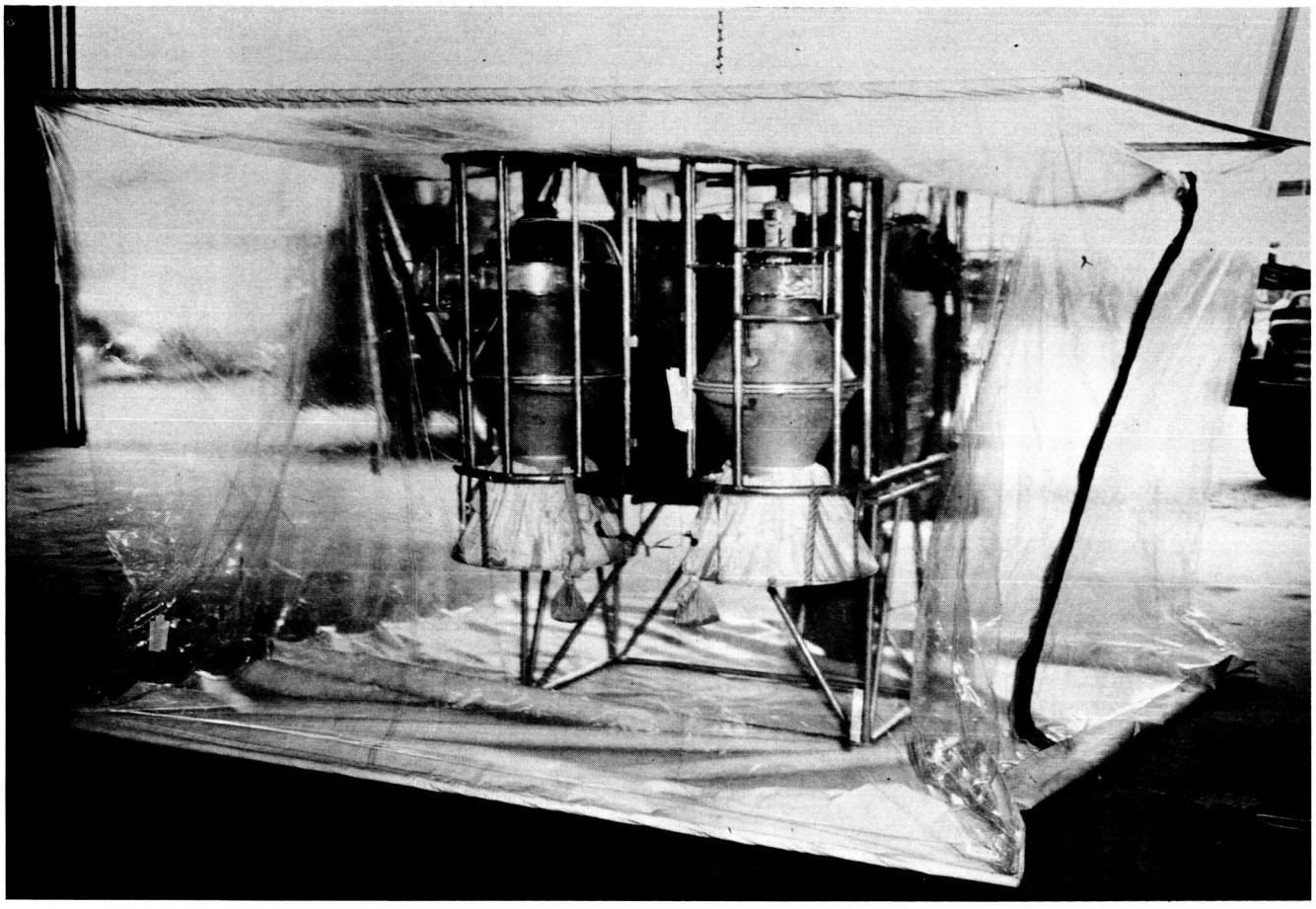


Fig. 4. Assembled payload immediately prior to flight. After removal of paper wrappers from sampling units and attaching units to gondola, payload was stored overnight in a vinyl-hypalon chamber filled with ethylene-oxide.

for as long as 1,000 minutes showed no greater loss in viability than replicate controls not exposed to such airflows. These experiments verified our assumption that any organism already present in the stratosphere would be able to withstand any "lethal" forces imposed by filtration.

Instrumentation

The gondola contained the following instruments and equipment:

A. *Control unit*, which opened helium valve on balloon at desired altitude to terminate ascent and initiate descent; switched blower motors on and off; fired squibs to jettison covers and shrouds; fired squibs to release spring-loaded sealing gates.

B. *Barocoder and 5-watt transmitter*, which telemetered altitude; was used as homing station for tracking aircraft; indicated functioning of blowers.

C. *Flowmeter recording unit*, which measured meter revolutions, inlet and exhaust air temperatures, and pressure drop across filters; recorded information on synchronized film.

D. *Tilt switch*, which released and destroyed balloon upon impact to prevent damage to payload by dragging.

E. *Power supply*: main power supply was 28 v dc with a capacity of 135 amp-hour.

Flights were monitored from a ground control station; a record of the functions was obtained by a camera mounted in the instrument pack. Tracking aircraft spotted the impact zone and directed recovery vehicles to the site by radio.

Flight Descriptions

Our primary intent was to elevate a sterile sampler into the stratosphere; to filter a large sample of air without contaminating it with fall-

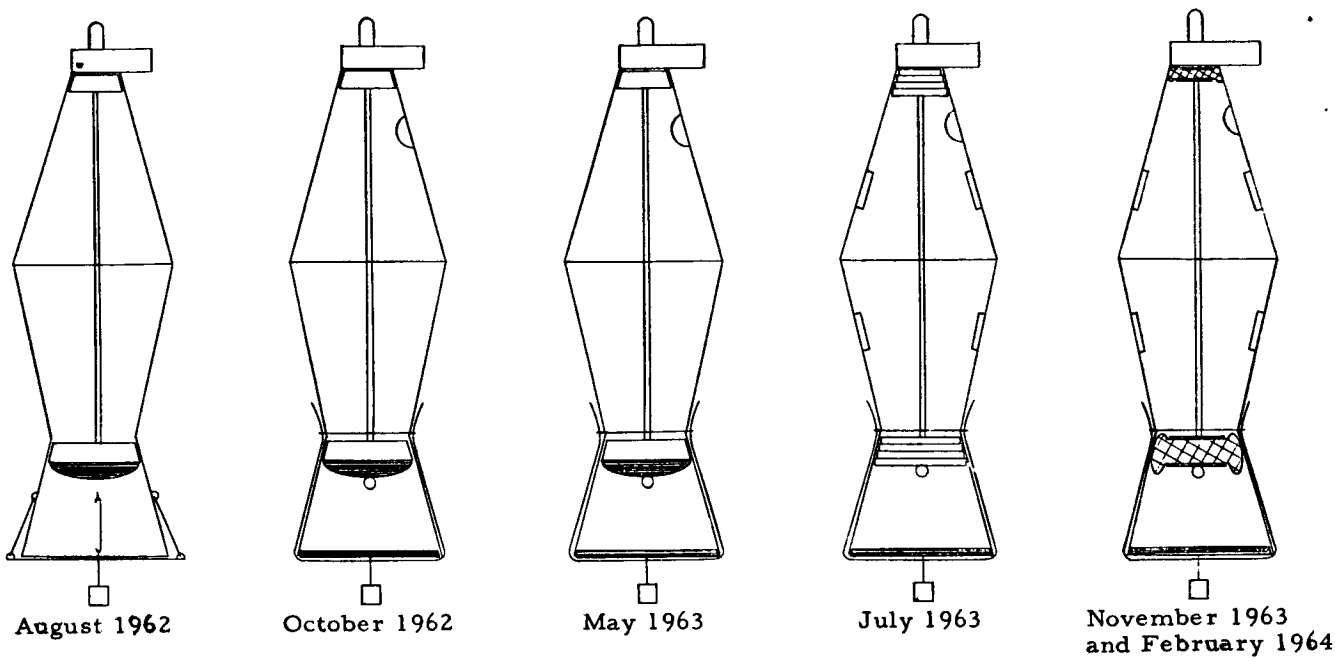


Fig. 5. Schematic drawings of modifications made on stratospheric biological sampler units during course of program.

NASr-81: *August 1962*. Seals: metal pans, polyurethane gaskets. Dust cover: aluminum sheet held in place with wires. Balsa wood nose cone over sealing pan. *October 1962*. Dust covers: held by nylon shroud cinched in place. Pressure equilibration port protected with absolute filter. *May 1963*. Seals: metal pans with silicone rubber gaskets. Metal spinning replaces balsa wood nose cone.

NASw-648: *July 1963*. Seals: plug made from overlapping sheets of polyurethane and seated against silicone rubber throat. Internal diffusion contamination controls introduced. Nose cones eliminated. *November 1963 & February 1964*. Seals: Gauze cotton plugs held in place with aluminum discs and seated against silicone rubber throat.

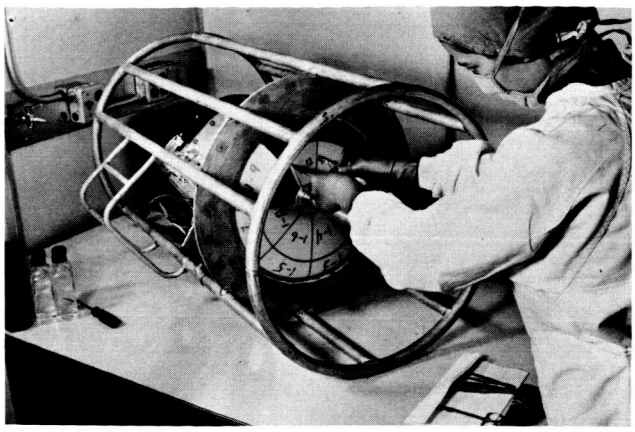
Original drawings presented in Final Report No. 2363, Contract NASr-81, 31 December 1962.

out from the balloon and gondola; to seal the exposed filter against subsequent low altitude and ground-borne contamination; and finally to culture any viable material trapped on the filter. Since a number of the concepts were being tested for the first time, Flight 1 served as a feasibility study, in which we could ascertain the mechanical reliablility of the equipment, the effectiveness of our anti-contamination precautions, and the suitability of our analytical techniques. Subsequently, a number of modifications were made, based on what we had learned from the initial experiment. Changes in the equipment were described above; changes in the flight program and sampling missions were incorporated as follows:

During Flight 1, the unshrouded dust covers were jettisoned at 10,000 ft during ascent. During the subsequent flights, the dust covers and shrouds were retained on the units until they reached 80,000 ft. During Flight 1, we sampled three profiles of air: 65,000 to 45,000 ft; 45,000 to 30,000 ft, and 30,000 to 10,000 ft. A control unit descended open from 65,000 to 45,000 ft, but its blower remained off. During the subsequent flights we tried to sample two profiles of air: 86,000 to 60,000 ft, and 60,000 to 40,000 ft. Two units served as controls: One was programmed to sample for a few minutes during float in order to measure fallout from the equipment. The other had its mechanical sealing gates closed just prior to launch in order to serve as an indicator of total pre-flight and post-flight contamination. Two scenes of the launch sequence are shown in Figures 8 and 9.

During the 2 years of this program, six flights were made. In Figure 10 the various trajectories and impact sites are shown. This pattern, of course, is a function of the altitudes attained and the stratospheric winds encountered. Most of the air masses sampled arrived from the west, and had originated over the Gulf of Alaska 3 to 6 days previously. Interestingly, during two flights (August 1962 & July 1963) winds at the highest altitudes sampled were easterlies, with an origin over the Atlantic.

In Table IV we have summarized the quantitative results of the 2-year program. Although we tried to keep our techniques consistent, the very nature of this work prevented each flight from being a replica of any other. Consequently



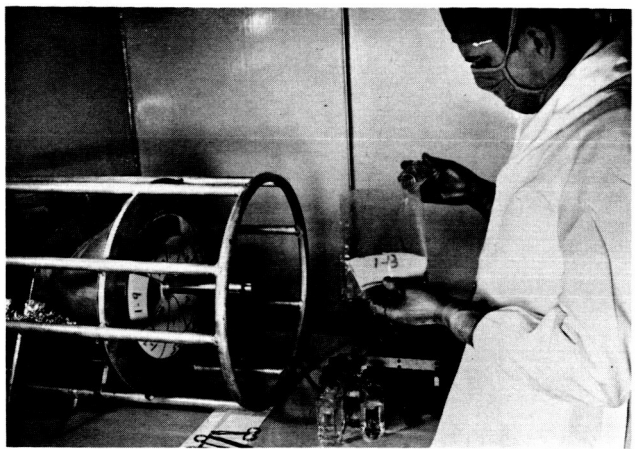


Fig. 6 *(top)*. Aseptic removal of filter pad from sampler after flight. Exterior of unit was chemically disinfected, and top spinning was removed in clean room to expose filter. Filter pad was then aseptically dissected into segments.

Fig. 7 *(bottom)*. Extraction of viable organisms from filter. Segments placed in sterile capran bag and sterile diluent added. Bag was then heat sealed and filter manually washed. Washings were cultured by membrane filtration technique.

we have provided below a detailed description of each flight performance, and our evaluation of the reliability of data from each experiment.

Flight 1 reached a maximum altitude of 65,000 ft and impacted safely in an oat field. All appeared satisfactory except that the sealing gates on the control sampler did not lock. There is also some question about the effectiveness of the gasketing on the other samplers. We are unsure about the extent of contamination from the formidable dust cloud that was created when the payload impacted.

Flight 2, after going through a perfect program, landed in a forest in the wilds of Wisconsin, knocking down two fairly large saplings in the process. The impact jarred loose the gates on three of the four samplers, permitting some contamination.

Flight 3 malfunctioned after the sampling sequence was successfully completed. Upon recovery in a fallow field, it was found that the gates on three samplers had never closed. The control was found locked as programmed.

Flight 4 performed according to program. It reached its scheduled altitude of 90,000 ft and descended gently, impacting into the side of a wooded hillside. All the gates were found locked. There is some doubt, however, about the ability

Table I

Incidental Contamination Contributed by Analytical Technique; Recovery Efficiency of Artificial Contaminants

Trial	Inoculum size	Recovery of organisms after extraction				Organisms, total recovery	Sterile control* total count/ft^2 filter
		1st	2nd	3rd	4th		
1	8,800	7,600	280	14	5	7,899	9
2	880	330	11	0	0	341	16
3	88	69	4	0	0	73	8
4	6	4	0	0	0	4	20
5	9,700	7,400	860	...	...	8,260	5
6	970	840	91	10	...	941	10
7	21,000	16,000	4,600	...	...	20,600	1
8	21,000	22,000	...	...	...	22,000	1
9	21,000	20,700	...	...	...	20,700	1
10	21,000	22,500	...	...	...	22,500	1

*Contaminants cultured after dissecting, extracting, and plating sterilized sheets of polyurethane foam.

Table II

Collection Efficiency of Polyurethane Filter (80 Pore)

Simulated altitude, ft	Filter thickness, inch	Linear velocity of airflow, fpm	Collection efficiency, %
30,000	No filter	500	0
10,000	1	377	50
30,000	1	420	60
30,000	1	503	78
45,000	1	542	91
45,000	1	818	99
60,000	1/2	900	>99
60,000	1/2	743	>99
90,000	1/2	734	>99

of the polyurethane plug to form a suitable seal when it has to seal itself at -50 C.

Flight 5 performed according to program. All functions were completed in proper sequence. The gates were sealed, and even though the payload landed in a well manured field, the cotton and gauze sealing pads seemed to have fulfilled their function.

Table III

Viability of Dry *S. marcescens* After Exposure to Airflows at Various Simulated Altitudes*

Simulated altitude	Exposure time, min	Airflow velocity, fpm	Viable count/cm^2
Sea level	10	0	2.08×10^3
		1,000	2.08×10^3
	100	0	5.46×10^2
		1,000	3.76×10^2
	1,000	0	1.28×10^2
		1,000	1.21×10^2
98,000 ft	10	0	4.37×10^3
		1,000	2.72×10^3
	100	0	2.35×10^3
		1,000	2.37×10^3
	1,000	0	4.70×10^2
		1,000	3.64×10^2

*Lyophilized powder uniformly deposited by aerosol (3-5μ) sedimentation onto sterile polyurethane filter pads.

Flight 6 aborted (see Fig. 11). After reaching a maximum altitude of 90,000 ft, some malfunction occurred (probably in the balloon) and the payload descended by chute. The samplers impacted open onto a frozen field without going through their sampling program. The only information gained from this flight was about the adequacy of our sterile controls.

Sampling Results

Flight 1. After the first flight, a considerable quantitative difference was evident between the samplers (Tables IV & V). Laboratory controls indicated that relatively few organisms were contributed by our techniques. The flight control (Sampler 2) indicated that some extraneous contamination had occurred, but since this unit had partially malfunctioned upon impact and since the gates were not locked, the counts may possibly reflect the malfunction. The extremely high count in Sampler 1 was surprising. The counts from Samplers 3 and 4 were not significantly different from the control.

Qualitative differences between the samplers were evident from gross observation of the culture plates. Subsequent isolation and detailed characterization procedures indicated that the filters exposed at different altitudes contained different predominating flora. The filter from Sampler 1 contained about 20,000 organisms, the majority of which were members of the pigmented genera *Flavobacterium* sp, *Brevibacterium* sp, and *Corynebacterium* sp. From this filter we also isolated a large number of white nonfermenting yeasts, some *Rhodotorula* sp, several thousand *Alternaria* sp, and *Cladosporium* sp. Although we searched carefully we could find no spore-forming bacilli, no actinomycetes, and no *Aspergilli* or *Penicillia* in this sampler. The flight control contained the same types of organisms as Sampler 1, although the total count on this filter was two orders of magnitude lower. The predominant organisms on the filters exposed below 45,000 ft (Samplers 3 and 4) were *Penicillium* sp. The few bacteria encountered were similar to those on Sampler 1.

As mentioned above, this first flight was essentially a feasibility study. Although the data are interesting, and may, indeed, be valid, they should be interpreted with caution. Considering the many sources of extraneous contamination, and the fact that after modifying our samplers we never again encountered such large numbers in a sampler that worked — we are now quite ready to admit that post-impact contamination with dust from the oat field accounted for this phenomenon.

Flight 2. The results from the second flight were considerably different. None of the filters yielded counts close to those observed from the first flight. We were encouraged by the low count in the float control sample, which indicated the low noise level attributable to balloon fall off. Even the relatively high count from what was supposed to be a sterile control (Sampler 4) was acceptable, considering that the sealing gates on this sampler sprang open after the payload knocked down two trees. We do not, however, feel that the slight difference between the counts from Samplers 1, 2, and 3 is sufficient to ascribe significance to the data from the second flight. Furthermore, the qualitative data also indicate the possibility of accidental contamination, although the predominance of *Cladosporium* in Sampler 3 was reminiscent of the first flight. In general, the types of organisms isolated from these filters were similar to the common variety of forest air and soil flora that we sampled at the impact site.

Since the maximum count above 60,000 ft was 128 organisms in 140,000 ft^3 of air, or somewhere in the order of $10^{-3}/ft^3$, and since a considerable number of these isolates were post-impact contaminants, the actual density in this air mass was probably $<< 10^{-3}/ft^3$. The same line of reasoning indicates that the actual viable count between 60,000 and 40,000 ft was $< <10^{-2}/ft^3$.

Flight 3. Unfortunately, the sealing mechanism malfunctioned during Flight 3. The plates were overgrown with common soil bacteria and fungi. The distribution of types indicated that the filters were thoroughly contaminated with dust during open impact. The recovery of only three organisms from the control unit that had been sealed prior to launch, however, indicated that

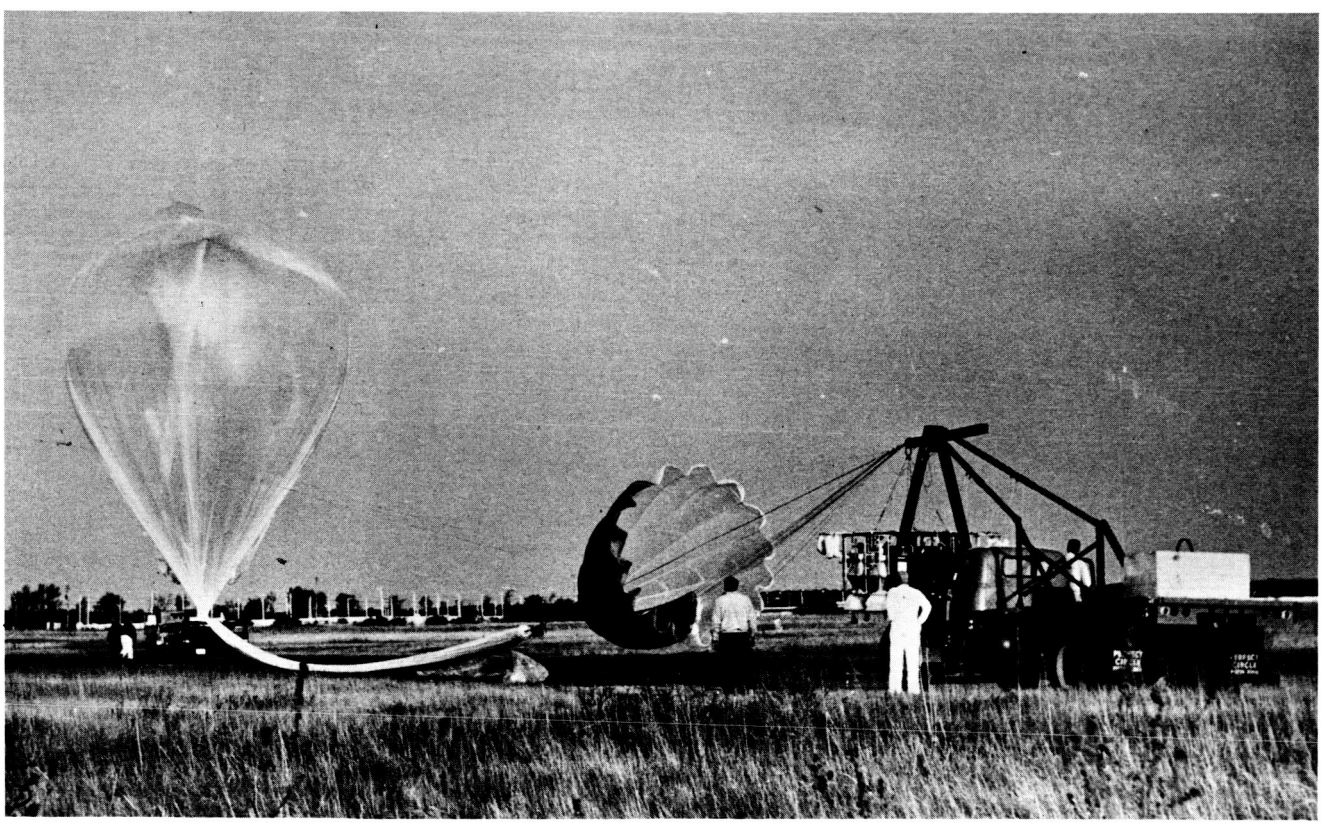


Fig. 8. Balloon launch. Note payload with shrouded samplers attached to truck. Balloon partially inflated with helium is being held down by mooring block. Uninflated portion of balloon is lying on clean polyethylene shroud (partly visible).

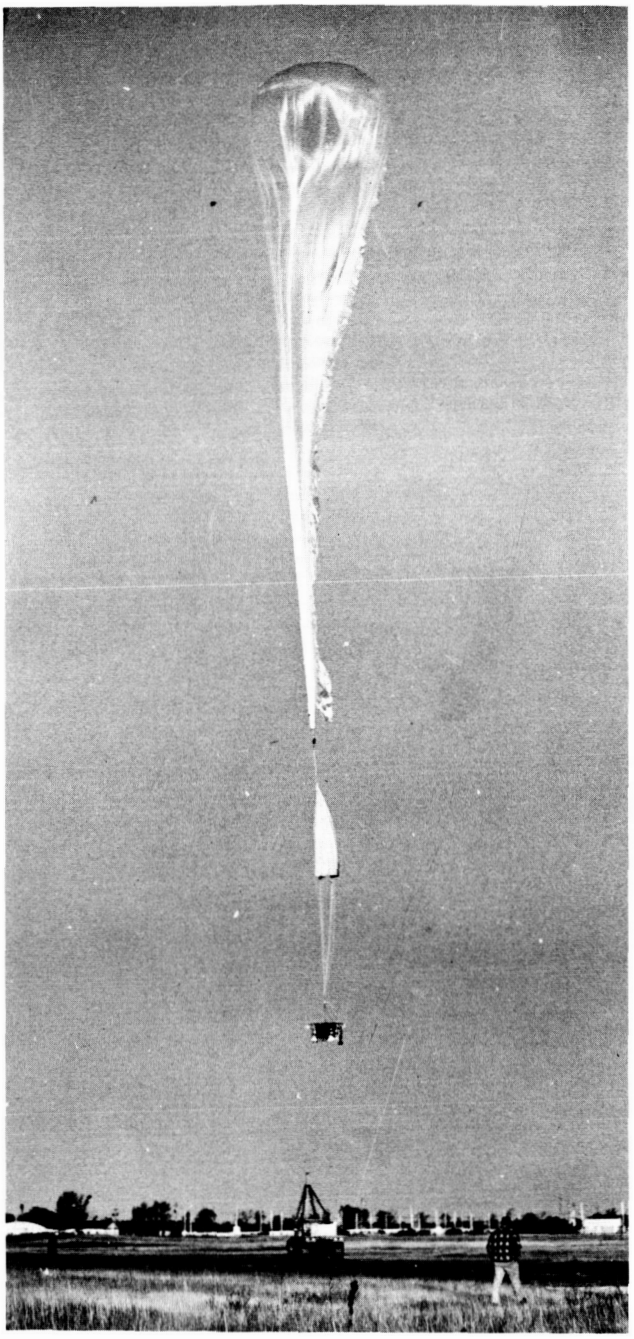


Fig. 9. Balloon launch. Payload starting its ascent 1 minute after release. Note flight train consisting of balloon, chute to slow down descent, and payload with shrouded samplers.

post impact contamination could be overcome if the mechanical equipment performed according to program.

Flight 4. The results from Flight 4 indicated that we were approaching our goal. There were only nine organisms on the filter pad of the control, and there was superficial agreement between the air volume sampled and the number of organisms recovered. *Cladosporium* and *Alternaria* molds were the predominant organisms on the sample filters, whereas gram negative rods and sporeformers were predominant on the control unit and the diffusion pads. We could not, however, attribute all of the growth from the exposed filters to a stratospheric source.

Qualitative examination of all the isolates from the diffusion pads and the sample filters indicated post impact contamination as a common source in certain cases, at least. Furthermore, the counts in the float controls were an order of magnitude higher than the count in the sample of 133,000 ft^3 of air when calculated on a density basis. We can make the following statement based on the results of this flight: If there are organisms in the stratosphere, their concentration probably does not exceed 3/1,000 ft^3 of ambient air, and the predominant types are probably *Cladosporium* and *Alternaria*.

Flight 5. This was a gratifying experiment, except that the control filter showed a few more organisms than the exposed sample filters. *Alternaria* and *Cladosporium* were again the common isolates from the high altitude samplers, whereas there were none isolated from the float control and the impact control. The commonest isolates from both the control unit and the diffusion pads of the other three units were gram negative rods and sporeformers indicating some post impact contamination. On this basis we can say that during November, at any rate, in an air mass which originated 5-1/2 days previously in Alaska, the microbial count was probably less than two organisms per 10,000 ft^3 of ambient air, and the predominant types were *Cladosporium* and *Alternaria*. This was not yet a perfect trial, but we were approaching a signal to noise ratio that made sense; we had reduced the background contamination (from preparation, storage, fall out, leakage, impact, transport, disassembly, and analysis) to such a

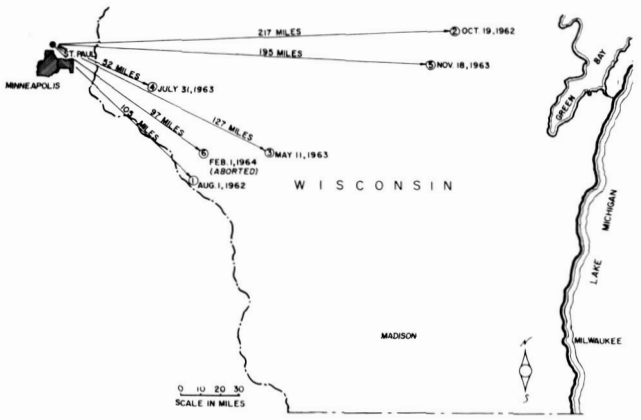


Fig. 10. Trajectories and impact points of six balloon flights. Flight numbers are encircled.

Table IV

Quantitative Bacteriological Results

Flight No.	Flight Date	Altitude profile sampled, ft x 10^3	Volume, ambient ft^3	Organisms recovered from sampler filter: Bacteria, yeasts, & Actinomycetes	Organisms recovered from sampler filter: Molds	Remarks
1.	Aug. 1, 1962	65-45	60,000	13,000	8,000	Gates locked?
		65-45	Control	240	64	Closed-not locked
		45-30	8,800	120	370	OK
		30-10	6,000	56	56	OK
2.	Oct. 19, 1962	88.7-60	140,000	106	22	Closed-not locked
		88.7	4,000	45	15	Closed-not locked
		60-40	20,400	140	56	OK
		Control	...	230	46	Opened after impact
3.	May 11, 1963	86.5-39.9	122,000	30,000	200,000	Impacted open
		Control	...	2	1	OK
4.	July 31, 1963	86	2,550?	45	53	OK
		86	4,530?	57	80	OK
		73.5-40	133,000	120	190	OK
		Control	...	5	4	OK
5.	Nov. 18, 1963	86	4,080	6	1	OK
		86-60	97,170	9	8	OK
		60-40	17,000	9	1	OK
		Control	...	21	0	OK
6.	Feb. 1, 1964	Flight aborted	...	300-2400	17,200	Impacted open

Table V

Qualitative Microbiological Results

Flight No.	Flight Date	Profile	Predominant types isolated from filter
1.	Aug. 1, 1962	65K-40K	Yeasts; pigmented diphtheroids; gram+ and gram- rods; coccobacilli
		40K-10K	*Cladosporium & Alternaria* *Penicillium; Cladosporium; Alternaria;* diphtheroids; gram+ rods; yeasts; diphtheroids
		Control	Gram+ rods; yeasts; diphtheroids
2.	Oct. 19, 1962	88.7K-40K	Micrococci; *Aspergillus; Cladosporium* diphtheroids
		Control	Actinomycetes; micrococci; *Aspergillus; Cladosporium;* diphtheroids
3.	May 11, 1963	Malfunction	
4.	July 31, 1963	86K-40K	*Cladosporium; Alternaria;* miscellaneous molds; spore-forming rods; gram- rods
		Control	Spore-forming rods & gram- rods
5.	Nov. 18, 1963	86K-40K	*Cladosporium; Alternaria;* gram+ rods; diphtheroids
		Control	Gram- rods; spore-forming rods; diphtheroids
6.	Feb. 1, 1964	Open Samplers	Gram+ rods; spore-forming rods; diphtheroids; micrococci
		Control	Gram+ rods

low level that if there were as few as five to ten organisms per 10,000 ft^3 of stratospheric air, we would be able to detect them easily.

Flight 6. This re-introduced us to the laws of probability and the concepts of humility. It was not a complete loss, however. The control unit, which had been closed prior to launch, had only three organisms on its filter pad, again demonstrating our noise control. The usefulness of the diffusion pads was demonstrated insofar as the organisms cultured from them verified quantitatively and qualitatively that the growth from the filter was obtained from a post-impact source.

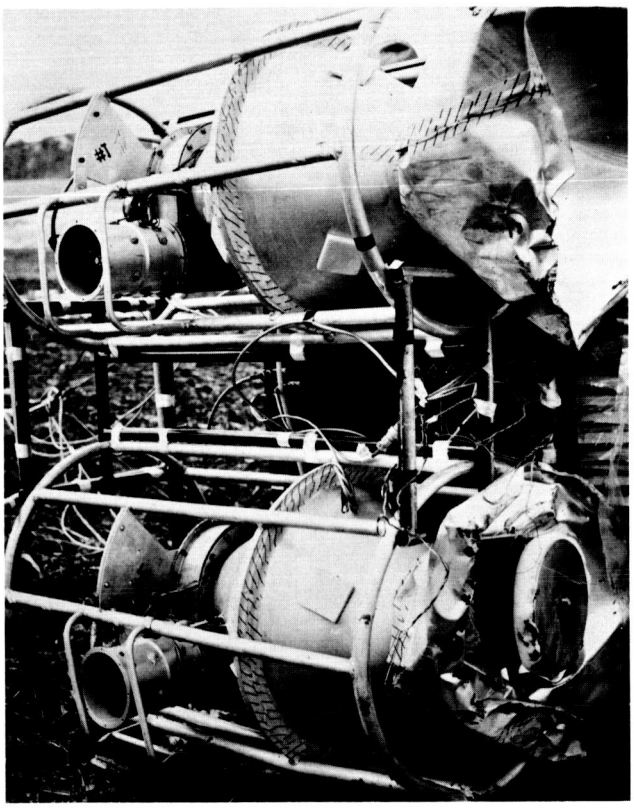


Fig. 11. Impact damage after aborted flight No. 6. Force of impact was sufficient to bend and break tubular aluminum frames.

Discussion - Conclusions

The successful prosecution of this type of research requires an appreciation and coordination of effort in at least three major areas: 1) flight operations, 2) mechanical engineering, 3) environmental microbiology.

We are fairly well satisfied with our balloon operations. We did successfully launch, fly, and recover five balloon-borne probes consecutively, and had only one serious mishap (attributable to a flight malfunction) in six trials. Even so, the payload was recovered after the last flight, and can be renovated for further experiments.

The engineering problems we imposed on our designers were rather formidable. We wanted a sampler 1) that could be autoclaved like a surgical instrument; 2) that could then operate in the temperatures and low pressures of the stratosphere; 3) that would sample in the order of 100,000 ft^3 of air and then seal itself; 4) that would withstand the stress of launch and impact while still retaining sterile integrity; 5) that would work remotely, automatically, and reliably — and would do all this within strict budgetary and time limitations. Our samplers are fairly primitive, but they do fulfill these requirements.

From a microbiological point of view, our most serious problems were contamination control and contamination monitoring. The results of flights 4 and 5 indicate that we have made significant progress in this area. Our major concern now is monitoring the contamination fallout that results when we disassemble the sampler just prior to dissecting out the filter. We are fairly confident, however, that this difficulty will be easily overcome.

Considering the complexity of the overall operation, in which a single malfunction in either balloon operations, engineering, or microbiology can invalidate the whole flight, we are pleased to have ANY results. It is even more gratifying to have confidence in results that provide some quantitative and qualitative insights into the biology of the stratosphere. We think that the fairly consistent recovery of *Alternaria* and *Cladosporium* on every successful flight is of some significance, particularly since it verifies the isolation of these genera by other workers at considerably lower altitudes. We are now fairly confident that the microbial count above the tropopause was somewhat less than 1×10^{-3} and probably was less than 1×10^{-4} organisms/ft^3 ambient, at least in the air masses we sampled.

These conclusions, of course, do not rule out the possibility of higher counts or different types of organisms being recovered by other explorations. Unforturnately, the task of sampling the stratosphere is somewhat more difficult than sampling the Pacific Ocean with a Pasteur pipette. We do not yet know how many samples to take or how frequently to take them before we gain statistical validity. We do know, however, about the many sources of noise that can be misinterpreted as signal.

We are still unable to interpret the surprising results from the first flight to everyone's satisfaction. We have not been able to repeat these observations, but this in itself does not necessarily invalidate them. It is possible that on our first attempt we encountered a rare meteorological-biological phenomenon. On the other hand, sober reflection indicates that the probability of undetected error on our first flight was greater than we considered possible at the time.

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Discussion

Phillips — I have two questions, Dr. Greene, concerning your technique. One question is about the figure you gave for the survival of *S. marcescens* on paper filters. The question is, was the *S. marcescens* disseminated as an aerosol, or how was it placed on the filter? The second question concerns the control sample, one from each flight. To what extent was that a control? The control went up and down I'm sure; did it open and close again, or did it open without drawing air through it?

Greene — With regard to the first question about artificial contamination of the filters. We exposed sterile pads of polyurethane foam in a room in which we had previously generated a dry aerosol of lyophilized *Serratia*. Essentially, therefore, the pads were exposed to the fallout of 3 to 5 μ particles. This was determined by microscopic examination of membrane filters exposed simultaneously.

With regard to the question about controls. During the first flight, the control was a sampler identical to the others, except that it went through the flight program without acquiring any sample. During the subsequent five flights, the control was a sampler that was sealed just before launch, and ascended and descended closed.

Bruch — I'm a little lost by this term you keep using about sampling 100,000 ft^3 of air. Dr. Junge used the term, mixing ratios, in his presentation. He based the term on particles per cubic centimeter of air. Now is your sampling volume reduced to standard temperature or pressure, is this space that you are describing, and how does it relate to a mixing ratio?

Greene — Our terms are ambient air. The ambient air volumes can be converted arithmetically into standard terms by figuring out where they were acquired and then multiplying by the appropriate conversion ratio. However, we were sampling profiles that started at 90,000 ft and ended at 60,000 ft, so there is a continuous change in the conversion ratio. This is why, for simplicity, we used the term, ambient cubic feet of air. All our results are then comparable to each other, rather than to those of someone else.

Bruch — That still leaves me a little confused. The other matter that I am concerned about is that your reports indicate that on the latter flights your internal controls on a per-unit-area basis were showing more counts, more organisms, than the actual samplers on which you base your results. Would you explain how this comes about?

Greene — The "diffusion controls," taped to the interior walls of the samplers, were supposed to distinguish between organisms entrained in the high velocity airstream (that is, trapped during the sampling process in the stratosphere), and those which entered through leaks during impact or while disassembling (that is, extraneous terrestrial contamination). In theory, and upon experimentation, the concept is valid. Of the particles in the air stream, 98% are collected on the main filter pad — only 2% on the diffusion controls. On the other hand, extraneous contaminants are deposited equally on the diffusion pads and the main filter.

Unfortunately Dr. Bruch is correct, and we can only guess at the answer. In many cases, the counts on the diffusion pads per unit area were as high, and sometimes higher, than that on the main filter. We suspect that this is a result of our disassembly technique, in which any fallout would be more pronounced on horizontal (that is, diffusion pad) surfaces than on vertical (that is, main filter) surfaces.

Qualitatively, the organisms on the diffusion pads were different than those on the main fil-

ter. But the criticism is valid. Until we find contamination only on the main filter and none on the diffusion controls, we will be forced to report the results as we did today; namely, that the known stratospheric counts are less than a certain value, because the organisms we did find might all have been extraneous contamination.

Goetz — What is the size of the spores in terms of the Stokes' fallout?

Gregory — The *Alternaria* spores might be up to 20 to 50 μ long. They would probably not be less than 10 to 20 μ in diameter; they are club-shaped. *Cladosporium* might be from 3, 4 up to 15μ, but you may have captured the smaller ones at high altitudes.

Goetz — This would mean that the Stokes' fallout rate at that reduced pressure would be very large indeed so that such particles could only be brought up by diffusion and convection of even greater velocity.

Dimmick — We've done some work with spores of *Coccidioides immitis* in a dried state. Interestingly enough, Dr. Goetz, they have a Stokes' diameter of about 0.1 μ although they are 4 or 5 μ in size — a fact that puzzled us for a long time. These spores will float on water, but if you put them in a vacuum, and then suddenly release the vacuum, the spores will sink. Also if you try to collect these spores on a slit sampler or a cascade impactor you get very few spores. However, if you put a millipore filter in the sampler, you collect in large numbers. This shows that the spores are there. So by three different ways our data indicate that the spores are possibly hollow, and act as if they had an aerodynamic diameter much smaller than they appear to have.

Goetz — This is exactly what is to be expected: If they are hollow and/or of a shape which represents a much smaller value than (0.17 x d) for the ratio: [volume (or mass)/surface area], their Stokes' density, and thus fallout rate, could easily become less than a tenth of the equivalent solid sphere.

Greene — The *Alternaria* spore looks like a hand grenade, depending upon the species with which one is dealing. A *Cladosporium* looks like a droplet.

Gregory — Something is known about the experimental rate of fall of both these types. They are, I think, high. The rate for *Alternaria* is perhaps higher than we would expect in the stratosphere, but possibly the rate for *Cladosporium* is not. The rates are approximately what might be expected from Stokes' law, certainly not an order of magnitude out, as has been shown in connection with *Coccidioides immitis*. Both these two forms are exceedingly common in the atmosphere. They are both pigmented; they would be good candidates for surviving in the stratosphere. The *Alternaria* spores are so large that one would not expect to find them in quantity in the stratosphere.

Church — Referring to your slide, I don't recall your list of microorganisms in its entirety. Would you use anaerobic conditions and light in your growth detection studies?

Greene — Yes. We used a number of media and culturing conditions. During the first flights we cultured aerobically on Mycophil, Eugonagar, and tryptone-glucose-yeast extract (TGE), and anaerobically on thioglycolate agar. In the last two flights we used TGE exclusively because the counts were so low, and this medium seemed to grow everything we had found previously.

Atmospheric Collection at 130,000 Feet

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Abstract

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A small biological sampler has been designed to collect particles from the upper atmosphere (130,000 ft). This device is an air impactor, capable of continuous sampling for 12 to 24 hours. The aerosol is impacted on the previously sterilized surface of a moving drum, driven by a timing mechanism. This enables the sample to be spread over a large surface and to be calibrated with respect to the time of collection. The sample is retrieved, handled aseptically, and cultured using standard laboratory procedures. The instrument has been tested under simulated atmospheric conditions and evaluated.

The instrument was flown in the spring of 1963, on three successive balloon flights (125,000 - 135,000 ft) above the southwestern United States. A small number of microorganisms was indicated. The results of these collections will be discussed.

Author

The first biological sampling of the earth's upper atmosphere at 130,000 ft has been performed on three balloon-borne missions from April 8 to May 14, 1963. A small air injector impactor type of collecting instrument was flown for 24-hour periods each. The collections were retrieved and microorganisms cultured and assayed.

While the ubiquitous nature of bacteria and their ability to adapt to almost any terrestrial environment is an accepted biological generality, there has been an increase in the investigation of the ecological relationship of organisms in exotic environments. This is particularly true with the rising interest in the possible existence of extraterrestrial life, where other planetary surfaces might present extremely hostile conditions for life and its biochemical processes, at least as we know it on earth (1). For survival and reproduction, the most limiting physical conditions imposed upon microorganisms by our environment, are dehydration, radiation, and rapid and severe changes in temperature and pressure. A large fraction of microorganisms might be air-borne, and survive atmospheric conditions, especially the spore formers; some studies have been made of atmospheric bacterial population densities. Little is known, however, of the vertical distribution of aerial plankton at altitudes above a few thousand feet.

The settling rate for particles the size and density of microorganisms is extremely slow. From simple calculation, assuming a specific gravity of 1.2 to 1.5 and a size of 0.5 to 1.0μ, the rate of fall for an organism suspended in the atmosphere (at standard temperature and pressure) is of the order of a few millimeters per hour. Most microorganisms do not float freely, but are attached to larger dust particles. Dust particles of the 25 to 100μ size settle at rates of a few meters per hour. Atmospheric turbulence sweeps up these particles from the surface; they might be suspended for long periods of time. Large numbers of organisms in the atmosphere have been found in surveys (7), especially over populated areas. While the numbers vary considerably, depending upon where and when the sample was taken, in general, average values up to several hundred organisms per cubic foot are common at sea level to a few hundred feet above.

This cannot be extrapolated to higher atmospheres. Changes and severity in temperature, humidity, and radiation are lethal to most microorganisms. Winds and density vary from one region to another of the atmosphere, and meteorology of the upper atmosphere is not completely known or understood. Some controversy exists concerning the amount of vertical mixing and the degree of stratification. The bacterial population of the upper atmosphere will be learned mostly through empirical exploration.

The difficulty of performing upper atmospheric sampling has been the lack of methods for carrying instruments to high altitudes. Airplanes are practical only to 30,000 to 40,000 ft. Balloons are costly and somewhat unreliable, but they offer the only reasonable method of attaining instrument flights of long duration above 50,000

ft. Modern balloon technology and materials have extended the limitations of weight, power, and time aloft, all of which are constraints on sample collection. Until 2 years ago the highest altitude sampled in our atmosphere was from 60,000 to 30,000 ft in 1936 during the Explorer II balloon program in 1936 (5). From a collection volume of about a thousand cubic feet, Rogers and Meier retrieved about ten microorganisms in their culture. In 1962 two balloons were flown by Greene (2), one at 60,000 ft and one at 90,000 ft in which a total of 60,000 ft^3 were sampled. Precaution was taken for good control, but the qualitative and quantitative results were variable. While not conclusive, the results indicate the presence of a small number of *Cladosporium* and *Alternaria* in the upper atmosphere.

In 1963 the present opportunity was provided to sample at 130,000 ft. In addition to the intrinsic value of extending the exploration of the atmosphere, a second motivation came from the planetary exploration program. Most of the biological experiments being developed for the planetary landing, unmanned missions require a sampling device. A concurrent effort is devoted towards developing a pneumatic instrument to collect an aerosolized soil or dust sample from the surface of Mars. One problem that has been considered is the low atmospheric pressure expected on the Martian surface. From calculation based on spectroscopic data of the planet (3), a value of 10 mb might be the surface pressure. This pressure corresponds to our atmosphere at about 100,000 ft. The atmospheric pressure at 130,000 ft where this experiment was performed is about three millibars. The temperature is -23 C and the mean free path is about six feet. It was believed that this opportunity could be used to develop an instrument whose principles could be tested under simulated Martian conditions as well as used in an actual field test.

The balloon borne instrument consisted of a continuous sampling air impactor operating at high efficiency. The ambient air was drawn into the instrument, passed through a narrow slit at high velocity and impacted against a surface. Those particles with sufficient inertia became attached to the surface where they adhered due to adhesive and electrostatic forces. The experimental package was recovered after each flight, returned to a nearby laboratory where special precautions were taken to open the package, and to remove the surface upon which the organisms were impacted; organisms on this surface were cultured using standard laboratory materials. The entire laboratory operation was carried out in a previously sterilized glove box.

The instrument consisted of two joined hemispheres (Fig. 1); one contained the collection apparatus, the other contained the battery pack. This allowed for easy assembly and removal of the sample. The collection assembly consisted of a porthole, a motor blower to move the air, a timing mechanism, valves, and a sterile chamber containing the slit and a rotating drum (Fig. 2). The rim surface of the drum was overlaid with a removable paper strip used as the impaction surface.

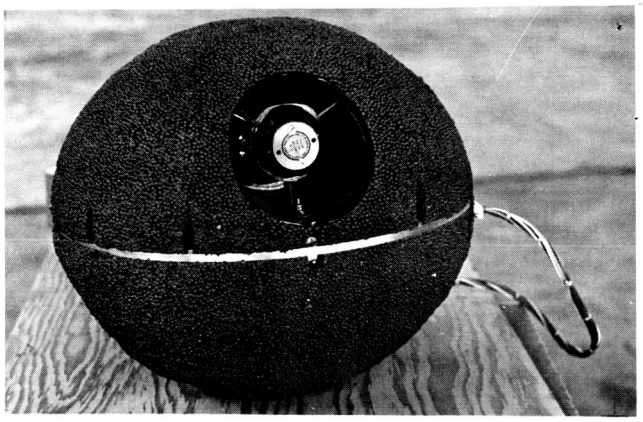

Fig. 1. Atmospheric sample collector. Opening in upper hemisphere is exhaust port of motor blower. Lower hemisphere with connected test leads contains battery pack. Hemispheres are shown joined as prepared for flight.

The blower unit was constructed downstream from the sterile collection chamber to prevent interference with the incoming particles. Outside air entered from a straight 1 inch, 0.5 inch diameter intake duct terminating in an impaction slit. The air passing through this slit was directed against the paper 0.75 inches from the surface. From the collecting chamber the air

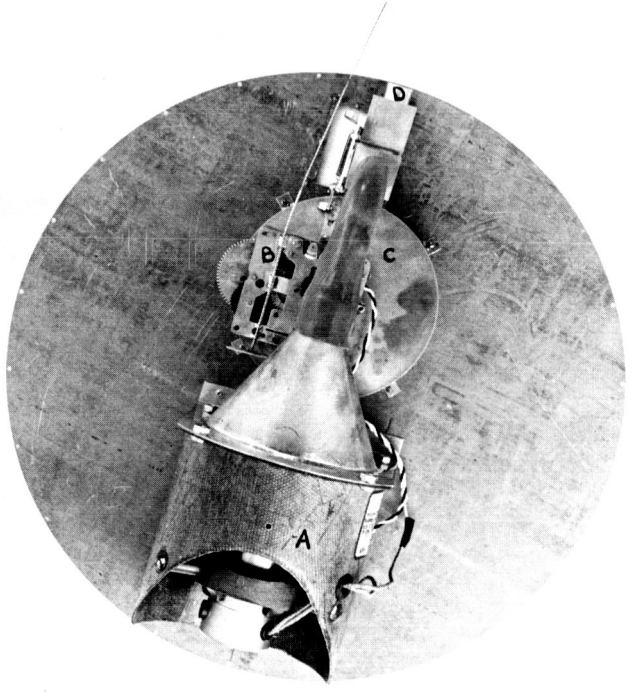

Fig. 2. Top view of collector assembly. Motor blower assembly (A) is exhausted to outside. Clock mechanism (B) times closing of valves, and drives collection drum surface. Drum is located in a housing (C). Intake port (D) can be sealed by a flapper valve located between it and drum housing.

was drawn into the blower and exhausted from the instrument. The impactor slit 0.10 inches x 0.68 inches terminated with streamline conversion from the circular intake duct. This configuration and this size were established using reasonable values for air density at that altitude (2.6 lb/ft^3), blower capacity, and particle size and density. A collection efficiency of 90% was calculated for particles larger than 0.1 μ and for density of greater than 1.0 (4). A drum housing held the impactor surface which was a 12 inch x 1 inch strip of filter paper (Whatman No. 42) wrapped on a 4-inch diameter drum with suitable cleats.

The drum with paper overlay was enclosed in a sealed chamber, passed by the impaction slit at a rate of 1 inch of surface per hour, and was powered by a spring driven clock mechanism. The clock was also used to start and stop the motor and to close spring-loaded flapper valves at both the entrance and exhaust ports of the collection chamber. To prevent contamination, closure of the ports was set for automatic operation at the termination of the experiment. The motor (24 v dc) was attached directly to the 4-inch diameter, four-blade axial-vane fan. The temperature characteristics of this commercial component were confirmed experimentally using a decompression chamber and a simulated atmosphere. Bearing surfaces were lubricated with suitable lubricants.

To power the motor, 18 silver zinc batteries (1.35 v) in series with a microswitch were used. Two batteries in series with a resistance heating element and thermostat were used to keep the batteries from freezing. The collecting assembly and battery assembly were each placed in one 16-inch diameter hollow hemisphere made of a low density resilient plastic material (Ecosil 5000) (Dupont). The walls of the hemisphere were 1-inch thick; the excess internal spaces were filled with the plastic. Total weight of the combined hemisphere was 20 lb. Ground test equipment was provided to test the assembled package. External leads permitted testing of the individual battery condition, motor blower operation, thermostat and heater, and the timing switches.

The air flow of the axial blower and slit was calibrated at various pressures ranging from 730 mm of mercury to 2 mm of mercury. This was done by mounting the blower on top of a standpipe 4 inches in diameter, 18 inches in length. The impactor slit was placed at the bottom of the standpipe, and the static pressure differential across the orifice was measured with a transducer. The transducer was calibrated using the deadweight of the diaphragm. Figure 3 is a curve showing the aerodynamic performance of this sampler. For 10 hours of sampling, a total of 1,000 ft^3 of air would be sampled at 130,000 ft altitude.

Tests were performed on several possible collecting surface materials to optimize collection. Discs 18 mm in diameter were cut to fit an experimental impactor previously described (6). The discs were sterilized and placed in the impactor. Using a bacteriological aerosol generator consisting of a nebulizer, a short mixing chamber, and a blower arrangement for moving the

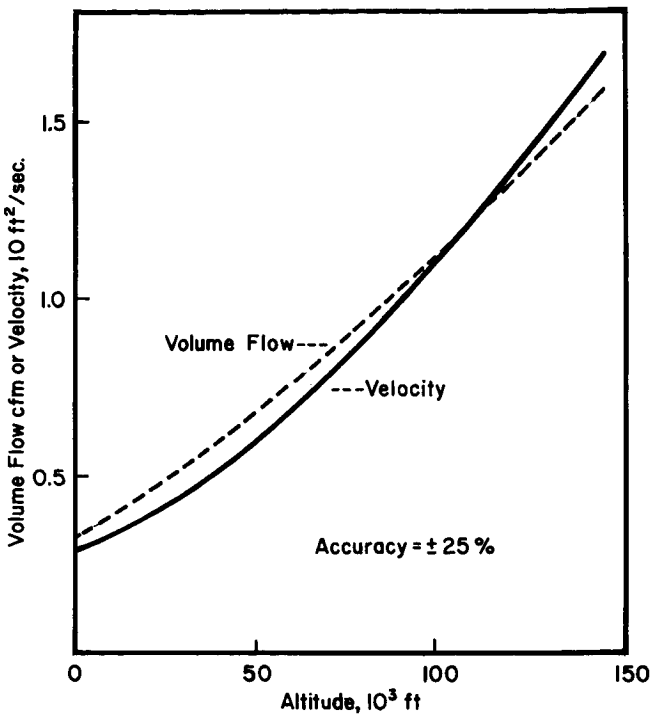

Fig. 3. Aerodynamic performance of aerosol impactor.

materials, the test material was inoculated using a standard stock suspension of *S. marcescens* as a test organism. The organisms from the aerosol generator were introduced into the impactor mechanism. The concentration of microorganisms in the aerosol and on the test material was assayed using a standard plating dilution technique. From these values the collection efficiency of the impactor surface material was calculated (Tables I and II).

Inoculated discs were diluted in 10 ml of sterile distilled water. Aliquots (1 ml) were plated using standard dilution technique. Two tests for each experiment, each in duplicate, were performed. The liquid impinger collected for 5 minutes in 40 ml of sterile distilled water. Aliquots (1 ml) were plated. Two tests were made, each in duplicate. From this, the determination was made of the density of organisms in the aerosol stream.

When the experiment was repeated (Table II), a value of 0.11% was obtained.

Establishing the high efficiency of collection for the filter paper permitted calibration of the experimental atmospheric sampler. In an experiment, a suspension of test organisms converted to an aerosol was introduced into the sampler in which filter paper (Whatman) was used as the collecting surface (Table III). The high efficiency for this instrument was due essentially to the high velocity air stream of the impactor. Airflow was approximately 150 fps (Table I).

Table I

Collecting Efficiency of Whatman No. 42 Filter Paper

Experiment	Impaction time	Organisms collected	Avg. min
Filter paper	1 min	5, 700	5, 700
Filter paper	2 min	11, 580	5, 790
Filter paper	3 min	15, 730	5, 240
Filter paper	4 min	22, 710	5, 680
			Avg. 5, 600

Stock suspension: 8×10^7 organisms/ml
Aerosol density: 5, 152 organisms/min
Surface collecting efficiency = $\frac{5,600}{5,152}$ = 100%

Table II

Collecting Efficiency of Dupont H Film

Experiment	Impaction time	Organisms collected	Avg. min
Dupont H film	1 min	10	10
Dupont H film	2 min	20	10
Dupont H film	3 min	10	4
Dupont H film	4 min	20	5
			Avg. 7

Stock suspension: 4×10^7 organisms/ml
Aerosol density: 2, 812 organisms/min
Surface collecting efficiency = $\frac{7}{2,812}$ = 0. 25%

The sampler was attached to the aerosol generator (Table III). Filter paper (Whatman No. 42) was used for the impactor surface. Each 1-hour segment was placed in 5 ml of sterile distilled water and agitated. Aliquots (1 ml) were plated with standard techniques for 48 hours in trypticase soy agar. Controls consisted of that portion of the paper that did not pass in front of the impactor.

Table III

Collecting Efficiency of Jet Aerosol Sampler

Segment	Total organisms/hr calc.
1	128
2	90
3	58
4	143
5	93
	Avg. 102

Control: 4 organisms/hr
Aerosol density: 99 organisms/hr
Aerosol impactor efficiency = $\frac{102}{99}$ = 100%

Two field tests were carried out on the California desert in still air to test the performance of the entire assembly. The results (Table IV) indicated a collection of approximately two to five organisms per cubic foot. This was reasonably within the range of expected results. The sample collections operated at a rate of 0.25 cfm.

Counts were made with standard plating techniques (Table IV). Atmospheric condition: still air. At sea level the volume flow of the impactor was 0.25 cfm. Two to five organisms per cubic foot were collected.

Extreme precautions were maintained in carrying out the biological part of this experiment. The paper impactor surface was autoclaved before assembly. Assembly was carried out in a glove box that had been previously sterilized for 6 hours with ethylene oxide gas. Following assembly the entire instrument was left in the glove box and exposed to the ethylene oxide for an additional 6 hours. All valves were closed and both ports were sealed with sterile tape. The tape was not removed until the moment of launch.

At the moment of launch the tape was removed, the entry port was opened and the timing mechanism started. Timing was set so that sampling was begun 2 hours after launch. By that time the balloon was stabilized at altitude.

An internal control consisted of retaining a segment of the paper free from impaction (Fig. 2). The drum was set so that a 2-inch portion of the 12-inch paper strip did not pass in front of the slit, and would therefore not be impacted by the air stream. This segment was treated in the same way as was the experimental impacted segment. In no case was the control found to be contaminated.

Upon retrieval of the instrument, all surfaces were decontaminated with formalin and 70% ethyl alcohol. The portholes were sealed, and the instrument was returned to the glove box. The hemispheres were carefully opened and decontaminated. The sealed chamber was opened using the sterile gloves; the paper strip, including the control, was removed with sterile instruments. The paper was cut into small segments that were transferred to culture broth tubes and culture plates. All tubes and plates were sealed with sterile tape before removal from the glove box. Samples of microorganisms were also taken from landing and launch sites, and from the balloon surface material for culturing.

Three types of solid culture media and three types of broth media were used. The solid culture was nutrient agar, trypticase soy agar as a general culturing media, and rose bengal agar to develop the molds. In scoring the results it was decided that only those organisms that occurred between the agar and the impacted paper segment (as indicated by a colony) could justifiably be counted. Any extraneous colony would be ignored; however, in no case was there any contaminant, not between the paper and the culture plate. These controls were extremely important in establishing the sterile handling technique.

The experiment was performed three times on three successful balloon flights. During Flight II, however, the motor mechanism of the collecting instrument malfunctioned and no organisms were collected.

From Flight I seven *Penicillium* molds were collected each on different plates; these organisms developed on the trypticase soy agar culture. The nutrient agar produced a single colony several weeks after collection of a yeast, saccharomyces. The rose bengal agar plates had no growth. Plates that were not sub-cultured were observed for 3 months. All colonies were subcultured onto new plates. In the liquid broth only the trypticase soy broth produced anything. While no quantitative result was possible from a liquid broth culture, only a single species of *Penicillium* was plated out. It must be presumed that only one organism was cultured from this.

Table IV

Field Tests

Segment*	Total organisms/hr calc.
1	75
2	30
3	210
4	80
	Controls 4
Segment*	**Total organisms/hr calc.**
1	25
2	20
3	48
4	38
5	28
	Controls 4

*Each segment refers to 1 inch of collecting surface (1 hr).

During the second flight, no organisms were found on the plates. One of the impactor-inoculated tubes of liquid Sabouraud media became cloudy, but it was not possible to culture any organisms from this.

From Flight III, five organisms were collected. Three were cultured from plates of trypticase soy agar (all on a single plate), and the other two were cultured on separate plates of rose bengal agar. All were *Penicillium* molds. No organisms were found in the liquid broth media. All organisms were sub-cultured. All plates and tubes that were not sub-cultured were kept under observation for 3 months for possible growth.

Conclusion

The most certain conclusion that can be made from these results is that either an extremely small number, or perhaps no microorganisms reside in the upper atmosphere. In these experiments at least 2,000 ft^3 of air were sampled, and only a total possible 14 organisms was encountered, maximally. While special precautions were taken, and controls were successfully maintained, bacteriological practice did not permit the statistical treatment of these small numbers. If these experiments were not so unique, they would have to be repeated many times to be meaningful. Nevertheless, it is quite apparent that while there may be no viable microorganisms existing at these altitudes, this experiment still permits the possibility of a very few. It can be said with confidence that *the number is of the order of ten or less microorganisms per thousand cubic feet of volume.* This number is consistent with other studies mentioned.

The fact that only one or two species of organisms were found may be significant. Both *Penicillium* spores and yeasts are known for their

extreme hardiness. They are small and resistent to drying, and might be expected to survive the harsh conditions of the upper atmosphere.

One problem with this kind of collecting technique is the possibility that the collecting material comes from the balloon surface. The payload was suspended several hundred feet below the balloon, the orifice was pointed downward, and sampling was not initiated until the balloon had come to equilibrium. The fact that there were 3 million ft^3 of balloon surface, however, that had not been sterilized would make one suspicious of any results, if the number of organisms collected was large.

The population density of the upper atmosphere can only be narrowed to small numbers. What these organisms are and how many there are, remain a problem that will take a good deal more investigation to establish.

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Discussion

Bruch — I am curious why you went to selective media such as rose bengal agar or to a medium of limited growth potential such as nutrient agar. It seems to me you would want a medium with broad growth potential.

Soffen — These were the standard techniques that the soils people were using at JPL at the time. They assured me that this was the ideal type of thing on which to grow certain molds.

Bruch — I have used rose bengal agar in the microbiological analysis of soils, but in terms of the minimal populations of organisms in the atmosphere rose bengal is the wrong medium to use.

Soffen — The last time I sampled, I only plated my samples on one media. However, it's undeniable that part of our results were on the rose bengal. On the other hand, growth may well have occurred had I put the sample on any of the other media. The probability of finding an organism is so low that the medium is almost unimportant in terms of the problem. Your suggestion is certainly right, although I plead ignorance for why I selected this rose bengal in the beginning.

MacLeod — Were you using any temperature or pressure trace on this?

Soffen — By pressure trace, you mean in terms of the gondola?

MacLeod — Yes, the environment that you went through.

Soffen — Yes, this is what a balloon flight looks like.

MacLeod — There is the possibility of reducing your air flow to standard . . .

Soffen — Oh, in any of these cases, there is the capability of reducing air flow to standard, but my device wasn't measuring intake or outflow. There was no flow meter attached. I used only a device that had been calibrated here on the ground under atmospheric pressures that we expected to encounter.

MacLeod — One of the important questions that we have to answer or look into is that of transport mechanisms, getting these organisms up into the atmosphere. In other words, if we find organisms there, we must have a good method for getting them into the upper troposphere, past the tropopause, and into the stratosphere. On these flights it might be important to do these calculations and include the . . .

Soffen — I should say that the motivation for performing in this particular way was that I thought I was going to be getting vertical distribution; as you can see I had an opportunity to fly on a flight that was going to be rising slowly, and was going to be up at high altitude for 24 hours. Initially, I had the idea of watching as a time

course of events, what took place on a different impacting area as this balloon rose. Difficulty was more engineering than anything else. There just are not motors that will function from the pressures we have on the surface to the pressures that exist at high altitudes, with the same degree of efficiency or anything like the same capability. This doesn't mean such motors don't exist; it just means that I didn't have one. And I didn't have the kind of research program that would go into funding such a device. We had our choice of flying or not flying; there was one altitude being selected. This wasn't my flight; I was piggybacking on someone else's flight. I was simply then stuck with the problem of finding a device that would work under the conditions where we'd be the longest. I changed in midstream and decided that clearly the thing to do was to take advantage of the fact that this was a unique flight at 130,000 ft.

Goetz — Isn't the efficiency of impaction and also that of the limiting orifice (which I understand was built into your system) quite low at the pressures that you encounter at pressures of about ten millibars?

Soffen — I misled you, Dr Goetz. It wasn't a critical orifice on this particular device. There was a critical orifice, of course, on the calibrating device. In fact, it was a commercial unit, but on this particular device the orifice was experimentally designed to operate optimally to get a maximum flow and at the same time the maximum number of organisms. The orifice was about a millimeter in size. This is hardly critical for the volume of flow. On one of the slides the velocity was plotted against altitude. The velocity was something like one quarter mach.

Goetz — At this altitude, shouldn't the impactor or slit actually be the limiting orifice? Was it a slit impactor?

Soffen — It was a slit impactor. It was limiting but it wasn't critical.

Goetz — In your balloon test, you have only the slit?

Soffen — That's quite right.

Goetz — But still, have you ever calculated what the impact efficiency is at these low pressures?

Soffen — The second flight was an attempt to find out in terms of a given organism. Now what is true for *Bacillus subtilis* of course is hardly true for any other organism, particularly something like *Penicillium* mold. On the other hand, one must realize that this was a limited program in the same sense. As such, I simply tried to find out for one specific organism what kind of efficiency I might expect. I was satisfied the efficiency was high enough not to bother changing anything, especially at that late date.

But your question is quite right. As a matter of fact, it brings out the point I was trying to make and have failed to make, that is, it's easy to read about other people's work; it's another thing to fly. It's difficult to build and to try to get meaningful results. It's somewhat shocking to find that there are so many people willing to talk about a project, but so few willing to perform. This is not an accusation of any one group; it's an accusation of all of biology because in fact balloons are being used all the time. Opportunities do exist; people are delighted to take an experiment along.

It's not expensive to build an experimental device. My clock cost $6.98. My airline ticket was expensive, but the motor cost $8.95. The slides are expensive, but the device itself is not. It is expensive to do it correctly; it is not expensive to try the experiment though. One's experience accumulates quickly up to a point and then of course it levels off and accumulates slowly from then on. What Dr. Greene is arguing with is that it's a cheap business. It's not a cheap business at all; it's just that getting some experience is not expensive and there ought not to be just one or two people in the country gaining this experience, particularly since I'm not in the field any more.

Moderator: H. M. Tsuchiya

Panel Discussion

Panelists:

A. Belmont
R. L. Dimmick
V. W. Greene
P. H. Gregory
C. E. Junge
N. H. MacLeod

▶ Belmont — Relatively little that has been said early in this conference has anything to do with the free atmosphere. I would like to make one cautionary statement.

First, I cannot stress too strongly that any measurements that are made of the atmosphere, whether at 30 km or near the earth's surface, must take into account what it is you are trying to compare. Scientists must see that there is uniformity of time and space scales. Lack of such uniformity is a very commonly overlooked error. For example, those who are concerned with the relations of allergy problems to air pollution, those who measure by putting their hand out the window and perhaps compare pollution at the laboratory with wind speeds, say, at the airport 10 miles away cannot obtain comparable data. The temperature at the wall does not indicate the actual temperature in an apparatus on the lab table. Obviously one must have carefully controlled conditions which are appropriately measured for the micro scale. Measurements of this type are difficult, if not impossible, to make.

The point is, one must recognize that there may be relatively large time and space variations in atmospheric measurements on all scales.

Second, as a non-biologist I am surprised that more use has not been made of modern electronic procedures for measuring. There are different ways of counting; there is a discrepancy between what is live, what is dead, and what is dormant. If one can measure some physical property using the latest techniques (and thereby define new terms) all of us can speak of the same thing.

▶ Gregory — I appreciate the invitation to this conference. The phenomena of biology of the atmosphere are well worth studying for their sake.

Let me confess my ulterior motives in space biology. First of all, phytopathology; I earn my living as a plant pathologist. That is where I chose. Second, asthma; this is more in the nature of a recreation which chose me. I am interested in particles in the atmosphere, alive or dead — alive and dead.

I won't go much farther at this stage beyond drawing attention to one possible implication of Dr. Goetz's observation on organic matter in the atmosphere. This has nothing to do with plant pathology or space exploration. It has to do with some of these "place" asthmas. Certain asthmas are associated (in the literature, at any rate) with certain localities. This association was noted by Storm van Leeuwen (1925) in Holland (A), perhaps 30 years ago. Many of these asthmas have never been explained. They are obviously not caused by ragweed, or anything else that we know. Could they possibly be associated with some of the substances that Dr. Goetz has found?

▶ Junge — As a meteorologist at this conference I have learned quite a bit about biology and other branches of science. This is always the case when one comes together with people from other fields.

First, I would like to emphasize that in the atmosphere insofar as transport is concerned we have actually three major regimes which can be distinguished to a certain degree, and which may be useful to distinguish in terms of transportation of organic matter.

One regime is certainly in the lower atmosphere over the continent. Here we have our primary sources of this organic material. Here a lot of cloud formation also goes on. This is the area where there are tremendous removal processes going on in terms of washout, sedimentation, and impaction on the earth surface or on trees, etc. This is an area where one would expect tremendous fluctuations in time and space.

Now, beyond 5 km (surprisingly low to my feeling as a meteorologist), apparently the regime changes considerably even over a large continent. Except in rather selective weather situations the washout becomes much less efficient because cloud formation no longer is of great importance in this area. There is a regime of high horizontal wind speeds, however. This means that there is a rapid drift across large areas. This is the regime where in time and space one can expect much less fluctuations (maybe by orders of magnitude less) and more uniform material.

Now the third regime is the stratosphere, which is quite different. There, sedimentation is the only removal process while turbulent diffusion carries the material upward. This results in a sedimentation-diffusion equilibrium distribution.

Now I want to make this point: one of the major interests at this conference is to learn what we find in the stratosphere and which of this may be of terrestrial origin.

The approach reported here by high-altitude collection with balloons, etc., is exciting and good. There's no doubt about it. But in view of the fact that these collections are rather expensive, one should also start doing some things which are more simple. I pointed out, that the upper troposphere is a regime where the distribution of organic and inorganic material is probably relatively uniform. We should make a much greater effort to collect in this area by more or less routinely flying aircraft, if possible, or by using specially equipped aircraft to obtain good solid data on the average composition of microorganisms in this part of the atmosphere. I have the impression from these talks that probably already in the upper troposphere near the tropopause, the number of species is tremendously reduced for reasons of size, etc.

Now, the composition in the upper troposphere controls the composition of the material which enters the stratosphere from below.

Not only should we know and establish frequencies and distributions for species in the upper troposphere, but also we should investigate rather carefully their sizes so that we know how they behave as physical particles in the sedimentation process as they enter the stratosphere.

If we know on a global scale the frequency in the upper troposphere, and if we know in addition, the absolute concentrations in these layers and their sizes (in other words, their Stokes' parameter of fall speed), then we can make reasonable guesses about their likely distribution in the stratosphere.

I agree that, unfortunately, we meteorologists do not have the precise knowledge of the mixing processes that we would like to have; still, I feel we can at least make reasonable guesses in this direction. These guesses would give us a reasonably good picture, at least within certain limits of what we might expect in the stratosphere to be of terrestrial origin. This kind of knowledge if it is properly organized can be obtained perhaps with not too much expense. One needs an aircraft which goes to 20,000 ft. I might stress that such an investigation should be based upon cooperation between scientists of various continents. Because each continent will produce a different composition of microorganisms in the atmosphere we have to find the average on a global scale [of] what enters the stratosphere.

I might also stress that we have good indications in meteorology that both hemispheres are reasonably well separated in horizontal mixing, so if one studies the northern hemisphere he probably does not have to study the southern hemisphere, and vice versa. There may be tremendous differences in the general composition between the two hemispheres. It might be interesting to obtain data on this subject.

With this information, obtained rather inexpensively, then, one could probably interpret more correctly whatever information one obtains from more expensive flights within the stratosphere.

Another point is the following: When I heard the papers on methods of collection I was struck by the absence of high-altitude aircraft. I have used such aircraft for scientific research in the middle stratosphere, around 20 km. Certainly such aircraft represent a fantastic opportunity which should be exploited.

I do not suggest using this method of collection as a substitute for balloon flying. On the contrary, both types of flight will give us much more comprehensive data than otherwise. The two methods supplement each other.With airplane flights at certain levels one gets a horizontal distribution, whereas with balloon flights one gets more of a profile; with both horizontal and vertical distributions one gets a reasonable picture of what goes on in the stratosphere.

▶ Greene—I should like to reemphasize Dr. Junge's remarks.

I have a feeling from this conference that from the 5-ft level researchers suddenly went to the 50,000-ft level. I'm not sure that we know everything that is going on at the 5-ft level despite the excellent work that is being done.

I agree wholeheartedly with what you have to say, Dr. Junge. Just one comment. Before one even gets into the upper structures above the tropopause, before he even gets up to 30,000 ft, there's a whole area within a mile of our own biosphere that we know very little about.

I suspect that an investigation of our own biosphere including the ground would be worth our effort. We would then be able to understand what extraterrestrial environments mean when we get into outer space. Otherwise we're going to be in the unenviable position of being unable to compare a sample from Mars with controls from earth.

▶ Dimmick — I'm no less than amazed at the gadgetry and the capacity of the engineering profession for some of these things; they seem almost impossible to me.

The thought strikes me that we in the closed ivory towers of research, when asked why we're doing something, usually say, "Well, we're just doing it out of curiosity," because if we do anything for a purpose we're accused of doing applied research.

What is the purpose of this upper atmospheric survey? It strikes me that primarily it might be curiosity; if so, then the engineering and developmental people might be accused of engaging in research out of curiosity. What I'm trying to get straight in my mind is really why you want to know about organisms in the troposphere. Naturally, it is a matter of curiosity to *wonder* if organisms are present in the troposphere.

Also, perhaps you're worried about the contamination of space probes by passage through the atmosphere (assuming that one could make them sterile on the ground) and thus carrying some of our own life to other planets (assuming that one can expect to find life on other planets). Now if this is really the problem, that is, that you want to make efforts to find life on other planets, the difficulties are magnified.

Whether you like it or not, when you talk about life on other planets you must exert some effort to define life and how it operates in our environment. Perhaps enough information already exists to enable us to understand life, but it is lost because of lack of communications. I have learned more from talking to various people here about what's going on than I would ever have learned from the literature. I'm sure that much of what's been reported here has not been published, and may never be. Perhaps we need to make up our minds as to whether or not this lack of communication is a serious problem. If it is a serious problem, maybe we require a miniature operations research in which people are centrally given the task of finding out what is going on, of putting together information, of asking people around the country for their advice. We need more than an occasional meeting.

▶ MacLeod — Being at the end of this very distinguished line of panelists, many of the things that I would like to have said have already been said.

I would like to extend your remarks, Dr. Dimmick, in terms of the cooperative communications, not only among individuals who are directly exploring the terrestrial biosphere, but also among those individuals who have contributed so well at this meeting from disciplines which we might normally think of as outside this area of investigation.

One of the problems that confronts the investigator is the lack of knowledge about mechanisms for transporting microbiological particles. We don't know too much about where the particles are and in what concentrations. The meteorologists do know quite a lot about transport and dynamics of air masses. We need to couple that information with our own sampling devices and analytical techniques. This might call for further development of instrumentation appropriately designed for sampling in not only the troposphere but also in the samplers that also would operate effectively in the unique environment of the stratosphere. Some of the papers have indicated that there are unique parameters and difficulties in defining the upper atmospheric environment — the stratosphere. One problem is the methods by which particles get into the stratosphere (if they do) and the mechanisms of fallout from the stratosphere.

There has been a problem, indeed, in clarifying the objectives for exploring this region of the biosphere. One course (as Dr. Bruch has mentioned earlier) is to obtain information in terms of sterilization and continued maintenance of sterility of probes going to planets, particularly to Mars.

My main impression from this meeting, however, is that there is a dynamic exchange between the products of the surface of the earth and of the atmosphere; we have just begun to realize the extent (not only in a horizontal direction but in a vertical direction) and the intensity of this exchange. Certainly, it is an objective of legitimate scientific interest to go all-out in an investigation of this region.

Tsuchiya — To summarize the comments of the panel members:

1. Dr. Belmont stresses the importance of the temporal and spatial scales which we must consider in interpreting the data that we get from various samplings. He also stresses the need for more modern physical methodologies. Finally, he suggests that rigorous definitions of life, death, etc., are needed.

2. Dr. Gregory is interested in viable and non-viable organic matter.

3. Dr. Junge mentioned the various regimes of transport and ways of losing biological systems from the atmosphere: over the continents, above 5 km, and then up to the stratosphere. He also mentioned the need for further information about the frequency and distribution of material in the troposphere. Dr. Junge suggests that possibly the organic matter, viable or otherwise, might differ over the various land masses. This should be investigated. He mentioned a number of methods for sampling which it might be well to investigate.

4. Dr. Greene spoke about the need for collecting materials at low levels so that we won't be carrying coals to Newcastle.

5. If I may put Dr. Dimmick's stimulative discussion in a capsule form, he, as a scientist, wants a sound definition of life.

We are concluding at least that there is necessity for interdisciplinary studies. It is unfortunate that we still use different words to mean the same thing, despite the fact that we all use,

at least here, the English language. The semantics pose a real difficulty. For example, I am sure that among many of the fluid mechanics people (that is, people interested in transport processes) the word "transport" as it is used by meteorologists would really be "convection."

General Remarks

Cole — This is not entirely correct. One could say part of the transport would be convection, but part of it also would be eddy diffusivity.

Tsuchiya — From meteorologists we have learned that the atmosphere is turbulent and the fluid mechanics people speak both about primary transport and secondary eddies. I merely want to point out that the semantics are such that we must be on guard in communicating with individuals in disciplines other than our own.

Mr. MacLeod referred to the need for understanding something of the transport mechanism of biological systems and the exchanges of particles and objects that occur both horizontally and vertically.

MacLeod — This is particularly in reference to the eddy transport that you were just talking about. I may not be correct in this, but I gathered that we may be talking about a unique kind of transport of biological particles. This would be mass transport as opposed to particle transport.

We have been talking about sedimentation, which is essentially a quiet-air phenomenon. Are these things closely coupled? Have we coupled these in sufficiently well-defined fashion?

Junge — Small particles can be treated as gases so far as transport in the atmosphere is involved. For particles longer than 1μ, that is, for all microorganisms, sedimentation becomes important. But so far as removal from the troposphere is concerned, washout and rainout are by far more important than sedimentation.

Tsuchiya — Dr. Greene, would you say that the spores of *Alternaria* or *Cladosporium* were by themselves? After all, *Alternaria* and *Cladosporium* are fairly good sized, aren't they?

Greene — We found these fungal colonies, particularly *Cladosporium*, growing on several plates where there was nothing else. This leads me to think that wherever these fungi were found, or however they got onto our filter pad, they got there by themselves.

We had several plates with a variety of microbial types, also. Sometimes as many as six or seven different bacterial and yeast colonies. On the other hand, several plates had pure cultures of molds only. On the first flight at altitudes from 45,000 to 30,000 ft and from 30,000 to 10,000 ft we had some pure cultures of *Penicillium* — absolutely pure cultures on some of the plates.

During flight 4 and flight 5, we found our *Alternaria* and *Cladosporium* almost on every plate; we found the fungi were practically by themselves.

Tsuchiya — Does this preclude the possibility that there may have been other organisms that were overgrown?

Greene — This is rather improbable, but does not preclude that possibility. It's just that we never saw them. We tried to give everything a chance to grow. We grew at room temperature. We grew in a neutral medium on these last few flights. We grew on a medium which would support a variety of saprophytes — tryptone-glucose-yeast extract — and we had nothing else growing on those plates, whereas on the control samples we had a mixture.

Gregory — Coming back to the interesting *Alternaria*, *Cladosporium* work, I think this is absolutely fascinating. The machinery is lovely; we are obviously at the beginning of a new epoch. I have been doing my best to think of methods for picking holes in the machinery. I don't think that the suggestion that there might have been other organisms present with the *Cladosporium* or *Alternaria* really worries me too much. The fact that some yeasts were associated with bacteria, I take it, is evidence that the sample consisted of a soil particle or a piece of vegetable dust in the atmosphere with a load of microbes on it. But probably the *Cladosporium* and the *Alternaria* arrived as single spores. They usually do at low levels, why not at higher levels?

Are there possibilities of other sources of contamination, Dr. Greene? Well, being as awkward as I can, I reflect that fabric is laid out on the ground, that the balloon itself, I take it, does not have a shroud. Does it have a shroud?

Greene — Oh yes. The balloon is never really laid out on the ground. The balloon is laid out on tables in the factory, many square feet of which have been sampled. The only things we can recover from them are the *Rhizopus* organisms which are associated with the starch with which we coat the balloon. We have never recovered a *Rhizopus* from a stratospheric sampler. We have hardly ever recovered any of the other fungi, that is, *Alternaria* and *Cladosporium*, from the balloon surface or from the starch.

On the runway the balloon is kept in its plastic wrapper until just before flight. Thus it does not come into contact at any time with anything except the hands of the people.

On several occasions we deliberately contaminated the balloon on the runway with several grams of micron-sized fluorescent particles. Then when the samplers came down again we examined our sampling filters to see if we had any of these fluorescent particles. There was no significant fallout from the balloon that could be recognized as such.

Bruch — Your microbial samplers and techniques of analysis have good sensitivity and can detect low numbers of organisms. The quantitative sensitivity of microscopic analysis is low, and you would need to recover many fluorescent particles for a positive response.

Greene — That's right. The number of fluorescent particles we apply to a balloon surface would be on the order of 10^{13} particles, and we didn't get them.

Bruch — What was the size range of these particles? Were they the same size as microorganisms?

Greene — One to three microns.

Perhaps Mr. McFarland, who did this work, would like to comment.

McFarland — We applied these particles to two separate flights — the fifth flight, which sampled, and the sixth flight, which did not sample. In both flights we detected roughly the same number of particles per unit area of filter medium. From this a person can logically conclude that whether you sample or whether you do not sample you arrive at roughly the same number of particles of this fluorescent type.

Furthermore, I might say that these zinc-cadmium sulphide particles that we used are a natural background in our laboratory. We have conducted extensive tests on the properties of powders; one of the powders is this zinc-cadmium sulphide, so that, as Dr. Greene pointed out, if one looks in a corner of the laboratory that hasn't been swept he will detect one to ten (something of this order) particles of the zinc-cadmium type per square centimeter.

I did attempt to analyze these results from the two flights statistically, but the numbers are so low that it is impossible to arrive at any significant figures. I would have to say that everything that we did detect would be contamination. That the particles don't fall off the balloon would be a logical premise here.

Gregory — I would like to make one other remark: from the ratios of viable to non-viable organisms normally found in the atmosphere it would be well worthwhile using a visual method on Dr. Greene's material. He would perhaps find far more, but dead *Alternaria* spores, and far more, but dead *Cladosporium* spores than viable spores of either of these molds. The spores are easy to identify microscopically, living or dead. He would not, I think, find maize [starch].

Belmont — I want to get back to Dr. Srinivasan's remark about transport. I'm not sure it was brought out in introductory lectures that transport to meteorologists is three dimensional, both horizontal and vertical, and can occur on all time and space scales. "Transport" to meteorologists can mean translocation just as to biologists, and may be applied not only to particulates but also to gases such as water vapor or ozone and to physical quantities such as momentum. I see no real difference between transport and mixing, except perhaps that the latter implies a closed circulation. There can be transport and mixing by all sizes of waves or eddies from molecular to planetary scales, in three dimensions.

Cole — One should always remember also that the particles that we are talking about do not follow the air motion. This transport that Dr. Wiin-Nielsen, and that Dr. Belmont were talking about, is transport of air or water vapor or gaseous material, but the particles if heavy are not going to follow exactly this air motion. The lighter particles will follow it better. This also always has to be considered, as it has been with some samplers, notably those used to sample ragweed pollen.

Dimmick — I don't see what all the fuss is about. Maybe I'm naive. It seems to me that you gave a man a problem. You expected him to see if there are organisms in the troposphere. He did what I think is a rather good job. Now you're surprised that he found a couple of organisms. I don't think it makes any difference, really, whether he did or he didn't find organisms. Although I frankly think he did. The fact that he found only a couple is not going to change your program at all; he didn't find them in tremendous quantities. You're still going to have to go up and sample and you're just as well off to say, "Yes, he did. He found these two organisms, so they're probably up there." And start from there. If you find out later that he's wrong, you haven't lost a thing.

MacLeod — I think the caution is generated, at least on my part, by thinking in terms of going to the planets. When we get high enough in the stratosphere there is the opportunity to get that which somebody wants to call "extraterrestrial." On all of our flights, therefore, our dynamic models must be rather well defined, taking into account past experience, so that we can have confidence that what is aloft is either terrestrial or extraterrestrial. The concern is not for methodology here; the concern is for a careful examination at every point along the line of the validity of the procedure and the analysis.

Dimmick — This seems like a preliminary experiment. You have some evidence that there are living things in the troposphere. You're going to have to do more work because your present results are not enough to satisfy you one way or another. They indicate, if nothing else, that the problem is exceedingly difficult. It's going to be expensive to solve. It's going to take a lot of work.

Gregory — May I come in again?

There's a lot on this topic I'm absolutely burning to make comments on. Dr. Dimmick says, "Is this matter serious?" Well, of course it is! I'm a plant pathologist. Let me ramble a bit.

We are concerned that we shouldn't contaminate Mars by sending space vehicles loaded with *Cladosporium* and *Alternaria* — at least not until we know that there are good *Cladosporium* and *Alternaria* there. That is I think a moral obligation; one can talk about morals here. One doesn't go to a completely unknown desert island and wipe out all the organisms there without knowing what they are. Perhaps if one knows what they are it might be worthwhile doing.

There's a still more serious aspect to this: one day space vehicles are going to come back. We want to have all the techniques ready for this event. Man is in a fortunate position, being the product of a long evolutionary series. Human beings are more or less accustomed to the organisms on our planet, and very few of our pathogens are outdoor airborne. Epidemics familiar to bacteriologists are mostly spread indoors — in subways, in theaters, in trains, in homes.

Now, it also is I believe generally held that one is more likely to develop immunity to a protein the more strange it is (at least some medical person correct me if I'm wrong). I have discussed this with immunologists. What about organisms different from the kind we're accustomed to, being brought from other planets and infecting us? Are we likely to all succumb to these? The answer usually given is, "No."

Well, that might be all right, but the evidence from immunology is a little thin to go on and I'm scared stiff. We only have to turn to *Coccidioides immitis*. Here is an organism which is highly infectious to man. Fortunately as a rule people don't suffer badly from it. But it also lives as a saprophyte. It is also readily airborne from the saprophytic state, and does not need sneezes to propel it into the air. We need only to imagine an airborne pathogen of this type, a little more successful saprophytically on this planet, a little more widespread in its host, a little more pathogenic, and you see why I'm scared stiff! Therefore, I do want to see a lot of research on the decontamination of space vehicles going out and coming back. It's all right to bring a virus like smallpox to the earth, and to bring plague, if you like, but don't bring something a good deal more successful saprophytically than *Coccidioides immitis*.

Solomon — I might echo your remarks, Dr. Gregory.

There certainly are some human diseases which are rather easily controlled if exposures are not overwhelming and progressive. Certainly plague is one of them. In areas where people are well nourished most people who contract plague have little trouble. If it comes to the individual in a fairly overwhelming inhalant form he develops pneumonic plague; the mortality rate for this is rather overwhelming. Coccidiomycosis also is a disease in which there is an interesting aspect: there seems to be a decided susceptibility to disseminated disease (and death) which selects different racial groups. Among Caucasians one can easily control the disease. Perhaps among Caucasians one in 5,000 or some order of this sort will develop disseminated disease, whereas among Filipinos, negroes, and other dark-skinned races a much higher percentage by orders of ten or more will develop generalized disease. Now whether or not organisms which might come in from the outside would find us as a more susceptible group than our normal saprophytic flora, I don't know; certainly it is something to think about and consider seriously.

Soffen — At an earlier session, maybe a year and a half ago, when Dr. Greene's work was first reported the question arose as to the source of these organisms. One of the explanations was, "Were we, in fact, bringing things up ourselves?" because vertical transport was questionable. A NASA representative said that tons of water are dumped into the upper atmosphere every time a rocket goes up. This water is simply ballast being unloaded. The question arose, "What is the source of that water?" It wasn't clear whether this was distilled water or whether it was water pumped out of a local lake. The water was merely weight.

The question I'm really bringing up is, do we know how much vertical bacterial transport we're responsible for in the form of balloon shots and aircraft that are being sent up there? Are we raising that number of bacteria with every shot? Do we know enough about this to be able to make an intelligent guess as to the rate?

I address this question to Dr. Bruch because he's in the best position to find the answers.

Bruch — The only comment I can make quickly here is that NASA has been accused of cluttering space not only with spacecraft that are still obiting, but also with ballast used to simulate dummy payloads.

Part of my skepticism about the collection of truly indigenous atmospheric microorganisms is that when I see that long balloon stretched out on the ground, I shudder at the thought of all those organisms that travel along with that balloon. Because of the differences in the sensitivity of assay techniques, I cannot accept fully the results of the tests with the zinc-cadmium sulfite particles. I want to expose this problem fully so that the audience as a whole can understand that our balloons may be seeding the stratosphere. From the evidence that has been presented so far I cannot reach the conclusion that there exists an indigenous atmospheric flora. It is possible that other space activities that have been described here are sources of the atmospheric flora.

From my own experience with spores, I know they can tolerate many deleterious conditions. One agent of the space environment that is quite destructive is ultraviolet. If spores are given any kind of protection against ultraviolet, they survive in nature for a long time. It would appear that *Alternaria* and *Cladosporium* are in the stratosphere in small numbers. I'm willing to go along with Dr. Greene that we interpret the data in terms that the numbers are less than a certain figure per so many thousands of cubic feet of space. If we can prove later that these values result from contamination of some sort, then we can correct the data accordingly.

One aspect I do want to point out is that the results from that first balloon flight by Dr. Greene received quite a bit of notoriety. I have literally taken it upon myself at NASA headquarters to stamp out the misconception that there are large numbers of organisms in the stratosphere as was first indicated in the results of that flight. I am eager to make sure that the data that come out of these flight programs are exact and true. If they can stand the test of scientific scrutiny, that is fine, but until we're certain of all the various

parameters that are impinging on this problem I'm reluctant to give the current results too much publicity.

Junge — I would like to make a few comments to this question which was raised by Dr. Soffen about contamination of our upper atmosphere by all our activities. This is, indeed, a serious question. It has been widely discussed in some committees which are concerned with the exploration of upper atmosphere. The general conclusion after reading these reports and thinking them over, I think, is that one should always keep one thing in mind, that is the huge volume of our atmosphere. Anything put into the atmosphere will finally be very diluted. If one really wants to contaminate the atmosphere with certain things considerable quantities of material are necessary.

In these committee reports, estimates were made, that only a much higher frequency of vehicles entering the upper atmosphere than at present is necessary in order to increase to any extent the amounts of certain constituents of the troposphere. The level of contamination will of course depend upon both the rate of input and the rate of removal of a particular constituent.

Tsuchiya — Dr. Junge spoke about sampling over the various land masses. I'd like to ask, "How much seasonal variation might there be over a land mass?"

Junge — We have no information on this question. I would say that the seasonal variation over a particular continent decreases rapidly above 5 km. The amplitude will be small and the phase will be later by one or two months, approximately, within the upper troposphere. There will still be some seasonal variation over a large continent because convection penetrates to the tropopause and the material is not carried away by winds so easily. But in general the non-frontal differences in the upper troposphere will be smoothed out to a large extent.

Tsuchiya — I am afraid of this business of averaging out. Could you give us some idea of the order of magnitude of the variation between the seasons in the upper troposphere?

Junge — This is hard to say because we have so little information. It would be entirely guesswork. I would say it is considerably reduced maybe by a factor of ten or so, maybe more. This depends to a large extent upon the size of the particle.

Tsuchiya — From the papers of Drs. Greene and Soffen we learned that the density was low. There are a lot of cubic miles above us in the atmosphere; therefore, just looking at the density values alone might be misleading.

Would you care to comment, Dr. Greene?

Greene — I think that there is no need to put any more quantitative label onto a figure when one says "less than." Less than one organism per thousand cubic feet of air is all we really know about it.

We'd be better off if we had a definite figure, for example, if we could verify that we found ten organisms. But thus far we haven't achieved this kind of confidence.

If we find something we can verify and if we know rather well where we found it and what it is, we will calculate this figure to something that is meaningful to meteorologists and physicists. We'll go to a lot of trouble. Right now there's no need to go to a lot of trouble because all we say is that we are establishing maximum limits.

Bruch — I just want to raise this point: the biologists have been trying to follow all the physical terminology that has been used in these discussions, and have been trying to understand the applications of Stokes' law. One aspect that bothers me is that nobody mentions the fact (maybe it is assumed and I am not catching it) that as we go above the earth the gravitational pull decreases. How does this affect the behavior of these atmospheric particulates? Is this implied in these density determinations that the physicists are discussing?

Goetz — It is of course true that gravity decreases very slightly at these altitudes and therefore one should expect the fallout rate to do the same. In the presence of an atmosphere the effect must be negligible compared to the deviations from the simple Stokes' law which occur when the mean free path of the gas molecules grows to the order of magnitude of the particles we consider here. The mean free path for the atmospheric gases at sea level pressure is somewhat less than 10^{-5} cm = 0.1 μ and should thus grow to the order of 10 μ in the pressure range (10^{-2} atm), I believe, we consider here. One knows from detailed studies that under these conditions the fallout rate for small particles is much increased as they, so to speak, increasingly "escape" molecular collisions which are effective to keep them in suspension. Hence it follows that the residence time of small particles becomes increasingly shorter, in accordance with the Knudsen-Cunningham correction of the classical Stokes' law. Since my experience with aerosols is restricted to the lowest layer of the biosphere, I have been wondering about the physical, as well as the chemical likelihood of supporting viable particles for any length of time at high altitudes except temporarily by the sheer accident of an upward jet. How likely would it be that we find live matter there? I can't help coming always to the crude conclusion that it is extremely unlikely that any viable particle should be there unless it has a very small *kinetic* size (as Dr. Gregory pointed out with regard to the geometry of these spores that have been found), namely, hollow shapes which expose much surface and have, therefore, kinetically, a small fraction of a unit density. Suppose one detects a specific gravity of 0.01, then, all would be different, as little bubbles or shapes acting like "parachutes" would have, of course, very small kinetic diameters, quite contrary to the little ideal sphere with unit density of which we always speak. Is it not true that most microbiological forms are not spherical?

Has anyone made detailed studies of the gravity fallout predicted from their size and mass? There are many factors in addition to be considered, particularly with regard to the sorptive interaction of the particle surface with the gaseous traces it contacts in the atmosphere which can modify the collision processes (accommodation coefficient) and thus alter the fallout rate.

I would like to mention an observation on the microbiological side here, as it seems pertinent: In recent years I consulted at a European research center for a program centering on the viability preservation of aerosolized bacteria and spores in a dynamically stable airflow at various humidity conditions, but particularly under the influence of sunlight (that is, not in the ultraviolet, but at the lower spectral limit of the biosphere above 3,200 A). Unless the air was extremely clean and artificially purified even on very short irradiation in a dynamic channel (patterned after our model in Pasadena), the viability was decreased to a remarkable extent even for spores. I speak of irradiation exposures at the rate of about 40,000 lumens/liter for less than 2 minutes which effected no temperature increase. Indications are that the presence of the oxides of nitrogen (traces of which apparently are ever present) produces highly destructive photochemical surface reactions on these organisms.

On the other hand, if the cells had a coating, they became amazingly stable. It took a long time, however, before the units could be induced thereafter to grow on a nutrient. Could not one make a few such experiments from this point of view, just to see if these particular spores would show that same sensitivity pattern? These tests, incidentally, were done with *B. globigii* spores and a variety of non-spore formers.

These are done at atmospheric pressure. Since the surface of the planet is the source of these organisms, however, isn't it necessary for organisms on their way to the tropopause to go along a photochemical "Via Dolorosa" (if I may call it that) of abundant contact with photons, oxides of nitrogen, and ozone? We don't necessarily need much ultraviolet.

I am simply concerned about the likelihood of an arrival and significant survival at these altitudes, regardless of what was present here, where the filter is placed. This was the real reason for my remark.

Soffen — I'd like to comment on something Dr. Greene may have forgotten in all this. He is currently working on the problem of collecting at pressures about which we're talking.

I was just asking for a finished statement about Stokes' law. I understand that Dr. Greene has tried to find out whether or not one could move organisms under low pressures in columns of air which would defeat the purpose if Stokes' law were still having its strong effect. In fact, Dr. Greene and his associates are able to collect with about the same efficiency, as though at ground level, by changing the character of the impactors. There certainly is an aerodynamic effect. I don't think we understand it; I don't know if anyone else is attempting a physical explanation. So far a phenomenon has simply been tested so as to validate the use of pneumatics on the surface of a planet that has such a low atmospheric pressure.

Goetz — My remark was focused not only at collection methods, but also at their presence in that part of the atmosphere where no one collects, let's say. Is a gas kinetically or dynamically likely to have particles of that size under these conditions without aerodynamic transportation?

Junge — May I comment on the likelihood of penetration. We do not understand the stratospheric vertical transport mechanisms well enough to make definite calculations and statements. Insofar as the estimates go, however, my computations seem to show that there is a likelihood that penetration goes quite a bit upward, even for particle sizes of the range of a micron or so, particularly for particles of low density. This is true even if the floating mechanism is different than one would expect for a sphere. I think there is good likelihood for penetration.

Goetz — Provided that they have this low kinetic density, due to their shape...

Junge — Yes, but even if there are spheres of 2 μ, one can expect penetration to 20 km or higher.

Goetz — At a density of one?

Junge — Yes, for a density of one.

Tsuchiya — May I ask a question of Mr. Beadle? Can you find fallout materials moving up and down? Up, specifically?

Beadle — There are people who could answer this question better than I. Of course, we have the advantage of a large amount of material up there, relatively speaking. We also know how to put it up there. The material is rather well pushed by the explosion. Levels, however, rapidly change in the stratosphere. For instance, in November 1962, Sr^{90} levels at 105,000 ft were ca. 90 dpm/KSCF (thousand standard cubic feet). In February 1963, following the largest Russian explosion and the largest to date, the levels rose to ca. 900 dpm/KSCF. We don't know if the Sr^{90} is going up or not, or if other particles are drifting upward or not. The Sr^{90} levels from the first half of 1963 are coming down (as of June, our last data). Strontium 90 is now in the vicinity of 700 dpm. From data gathered over many years of testing programs we conclude that Sr^{90} is descending slowly. There is still a large amount of material at these higher altitudes. Its descent appears to be very slow. We don't know the size of these particulates explicitly. They are estimated from a rather cursory examination of electromicrographs to be in the vicinity of 0.01 to 0.1 μ. These are metallic species, as oxides probably, so their densities are likely in the neighborhood of three, maybe five, depending upon what they are (which we don't know).

While I'm here, I'll answer an earlier question about seasonal variation. There is a seasonal variation at ground level, what we call a spring peak in fallout. This is evidenced by increases in the ground-level samples (air and precipitation), increases in milk concentration, etc., that many people are aware of from press reports. This level reaches a peak around June. People in our office can predict this peak rather accurately. These levels were a couple times higher than the year before, so that people were concerned about it. The levels are going to be as high this year, apparently, but they will fall off in the fall and winter. What they will be a year from now, I don't know, but they'll be somewhat lower than they are now, due to washout, etc.

This is due to the effects of spring rains and washout. The materials there, and the spring rains and washout phenomena bring the fallout particulates down faster.

Dr. Danielson mentioned a program of a year ago, which showed that the material is there, right at the tropopause. It will be brought down through these mechanisms and washed out. The levels during a storm, however, are much higher than those ahead of a storm front.

I have a question. A lot of tests in the Pacific intercepted the ocean or went below the ocean surface. In these tests, the fireballs were carried to or close to stratospheric levels. Is there a probability that biological life could be transported to the stratosphere by this mechanism? This could be a good injection possibility. Another possibility is that from the Nevada test site, from dirt — something on the periphery of the cloud that was not atomized, could rise on the cloud. Has this ever been thought about as an injection source?

Bruch — I would say that the fire or cloud that rises is not necessarily all due to radiation; a lot of it is due to thermal effects. From my knowledge of spores I would say that chances are good that they could tolerate the thermal effects and actually rise on that cloud and at least get into the higher parts of the troposphere. You indicated the stratosphere, and I am glad that you were thinking that high because this may be, as you say, an injection mechanism. Beyond that I am not prepared to comment. Mr. MacLeod, do you want to make that comment?

MacLeod — My thought is that maybe we're too late with this natural flora and fauna investigation of the upper atmosphere. I would think, after looking at the films that the fireball would look like an entirely possible mechanism for injection. These fallout particles that you're talking about, though, are substantially smaller, are they not, than the sort of thing that would be spore size? Did you say 0.1 μ and below?

Answer from the audience — Tenth micron and below.

MacLeod — Our figure was obtained at a considerably higher density. I wonder if these things are still up there. Is there a proposed mechanism for this, or have the diameters swollen? I'm sorry, the size is small enough now to stay there.

Junge — It depends very much on the particle size. Measurements seem to indicate that particles larger than 5 μ settle out in a quarter of a year or so from the stratosphere. What is left after awhile is in the size range below 0.1 μ. That is the size which carries most of the radioactivity after the first time has elapsed. These particles, which behave as a gas, reflect the circulation, and the exchange mechanism of the air in the lower stratosphere. The residence time of these particles in the layers between 20 to 30 km is perhaps three years or so. Apparently, the upper limit of residence in the stratosphere is 10 years no matter how high material is injected. This is for material which practically behaves like a gas. Larger material drops out faster. There is a likelihood that particles around a few microns can get up temporarily to layers above 25 km.

There is one other point: we do not understand right now the rather violent processes going on in the polar winter stratosphere. Dr. Belmont, however, can probably comment on that much better than I. Apparently these processes are surprisingly intense, much more so than we thought up to now. Once in awhile these processes may carry material up from lower layers, to layers of about 30 km, but not much beyond because even for 1 μ particles the fall speed then becomes too large compared to the vertical velocity of these ascending air motions.

MacLeod — Can I just say one more thing about this subject?

Here is the perfect example of this kind of interdisciplinary activity that we would like to build if we can. Here are a meteorologist and an atomic scientist working with stratospheric particles. We also are interested in this work. Some of the techniques that were outlined for the Atomic Energy Commission type of investigation are perhaps forerunners of the kind of instrumentation that is an engineering requirement for an investigation of the biological content of the stratosphere. Hopefully, this little quadrangle that just got set up here is something that will continue.

Comment from audience — I would like to add one thing to what Dr. Junge said. There is a committee on research aircraft (I don't know the particulars; I'm on the air pollution committee only as an alternate). This committee met for the first time in April 1964, to establish governmental interagency requirements for research aircraft. The committee is under the auspices of INCAR, I believe. This is, I gather, an organization that is going to find out *what* people are using *what* type of aircraft, for *what* purpose, and with *what* requirements in order to establish cooperative programs such as piggyback flights. In view of aircraft as the most reasonable means to sample in the tropopause, they will probably have some variety of aircraft available. You might think about this in sampling at these altitudes.

Cole — I should like to make one comment here about injecting material into at least the lower stratosphere. I don't know of any action of thunderstorms. Of course, there are many,

many such storms every year. They do get up to 50,000 or 60,000 ft. Here is a mechanism that breaks through the tropopause and into the stratosphere. It seems to me the storms will carry a lot of microbiological material.

Belmont — Occasionally there is stratospheric flow from the arctic almost to the equator, say, to the southern Caribbean, or central Africa. This condition may persist for many days, so there can be large-scale horizontal mixing and at the same time vertical mixing.

The question was raised earlier about the possibility of having some of these larger particles at a level as high as 100,000 ft or 10 mb. If these particles are brought up from natural processes, by either convection or strong vertical motions, the question isn't only what the fall rate is. As the atmosphere may experience prolonged vertical mixing at certain times and regions, particles may stay aloft long after a fall-out time based on fall-rate alone. Surely, large atomic tests release large amounts of material whose quantity is biologically significant and of which we are aware because it is so easily detected. However, the total mass is very small compared to particulates normally contained in the atmosphere which are carried up continually on a global scale by natural processes such as convection, thunderstorms, and larger scale disturbances. Vertical circulations around the several jet streams also help transport and mix materials from the troposphere to the highest stratospheric levels.

These natural vertical transport processes are far more important than any artificial rockets or other methods that we may use to bring materials to the stratosphere.

Goetz — It would be a matter of velocity. In other words, the vertical velocity component would have to be many times larger than the Stokes-Cunningham fallout rate. If one knows these velocities then naturally one can calculate the likelihood.

Belmont — Useful computations of these things, unfortunately, are difficult to make because appropriate measurements are usually lacking.

Greene — We recognize the work that you've been doing, Dr. Gregory. You're probably more familiar than any other person with what everyone else in the world is doing in this field. Has anyone experimentally attempted to lyophilize these organisms and to create what Dr. Goetz calls his little parachute effects? These have large surface areas and they have low densities. Has anyone tried to actually put them into simulated altitude chambers to see how long it takes the organisms to fall out or to see if Stokes' law applies? I recognize that this can be calculated; I am just wondering if anyone has done this experimentally.

Gregory — I wouldn't know. I don't think it's been done with lyophilized particles. All the work has been done with ordinary air-dried particles.

Dimmick — All the work we did was with lyophilized spores. When they were dried they were quite different than they were when they were lyophilized. Now, it seems to me that a particle starting out from the earth's surface and going up would first freeze. If it continued to be carried up it would then start to lose the water by sublimation. In essence there would be a freeze-dried particle.

Gregory — I think that answers the question.

MacLeod — I want to ask Dr. Belmont or Dr. Junge a question.

You were talking about the turbulence in the polar winter. What is your means for sampling in this area? Is this through some means of instrument in the air mass or something else?

Belmont — Insofar as meteorological parameters go the sampling is strictly by radiosonde measurements, which are unreliable at highest levels as we know. Radiosondes constitute a poor network with nothing over the oceans.

MacLeod — Is your radiosonde going up into something like the highest level of the stratosphere? What would this be?

Belmont — I meant the highest level that we reach with balloons, normally 10 mb/30 km.

MacLeod — This, in the arctic would be well into the stratosphere?

Belmont — Yes. The tropopause there is near 10 km, and we have data up to about 30 km.

Cole — There seems to be a discussion here about how to get these particles into the stratosphere; the question seems to revolve around how an air mass moving up can, you might say, drag the particles with it. This all implies treating the air as a continuum; I wonder if we shouldn't think a little bit about the fact that possibly we're getting to the region where we should not treat the air as a continuum, but as a collection of particles. I can't remember, frankly, what the mean free path is at 10 mb. Surely somewhere there is a point where the mean free path is much larger than these particles about which we're talking. The probability surely exists that one of these particles could continue to get hit on the bottom and keep getting pushed up. This explanation might account for having a small amount of such particles much higher than we could estimate if we treated merely Stokes'-law drag and that sort of thing in the results.

Steinberg — So far nobody has mentioned one possible mechanism for keeping a particle afloat once it's up there. This mechanism in some cases may aid in getting the particle a little higher. This is the so-called radiometer effect. Of course, there are a lot of if's in this particular effect. About three years ago we were interested in keeping particles afloat in the upper stratosphere, and wanted to know how much influence the so-called radiometer effect would have. I regret that I cannot remember all the details now. The data

showed a lot of if's which one must take into consideration: if the particle is the correct size, somewhere around the 5 μ region; if the particle is at an altitude where the mean free path is greater than the size of the particle; if the density is low enough (one or less); if the particle has on its surface a sort of insulating material where one part of the, say, sphere, is darker or able to store more energy than the other side so there is a gradient across the particle. If these conditions exist there is enough energy in this field to keep a particle afloat. Now, of course, we're talking about living organisms here; I can't see normally getting a gradient across a spore, but some deformity or discoloration on the spore itself may cause a temperature gradient across the spore and thus energy may be obtained for keeping the spore afloat.

This is something to think about.

Junge — I would like to comment on Dr. Cole's question. Sedimentation can be treated correctly even if the particles are small compared to the mean free path length of the air molecules. Correction factors enable one to treat this case as if the air is still a continuum, so that the combined effects of sedimentation and also turbulent diffusion can be calculated correctly up to altitudes of 50 km and more.

Gregory — I don't know whether or not the spore in question is the right size. We do know a group of fungi which have spores 4 to 8 μ diameter, roughly spherical, of which half the sphere is black and the other half light. (Some of the smuts of cereals and grasses.)

May I ask a question? I would like you people to list for me the various methods by which one can experimentally determine the rate of fall in air of particles of the size in which we are interested. I know one or two methods, but I would very much like to take away with me a list, so that I can try some methods that we don't use. We do use the Galileo method in an artificial temperature inversion.

Dimmick — You can use the very simple method of a stirred settling chamber. This is rather inexpensive and surprisingly accurate if used correctly. I've never actually used it under reduced pressures, but I don't see why this wouldn't work.

Gregory — What do you do?

Dimmick — This is in essence a box with a fan. You put an aerosol in it. Then you need a vertical velocity of air from that fan sufficient to loft the biggest particle you're interested in to the height of your chamber so that you maintain homogeneity. Then you can count the number of particles as a function of time or you can look at the particles by light scattering (which is the easier way of the two) and plot the light scattering decay as a function of time. From this and Stokes' law one can calculate the particle size. If you have a homogeneous aerosol you'll get a logarithmic decay.

Gregory — These seem to be the methods for determining the rate of fall: There is the Galileo method like dropping an object down the Tower of Pisa; you drop the particle down a column of still air, preferably with a temperature inversion to damp out vertical convection. That can be done by either timing the spore fall with a microscope or by using photography or some other method.

Centrifugation is another method, using the instrument called the conifuge.

There have been various experimental methods for determining the fall of *Lycopodium* spores at ground level. Values vary from about 1.6 cm/second to 2.2 cm/second.

Another method is to find the velocity of an ascending column of air at which the spores balance. That's a difficult method. I don't like most of those methods; I'm very glad to hear of this stirred settling chamber.

McFarland — Possibly for determining the Stokes' diameter of spores, the most appropriate method would be settling in a liquid, from which the suspension of spores would be floated on the surface of a liquid using either centrifugation or gravity settling. I realize that one of the deficiencies of this method is determination of the actual density of particles. There are methods for getting around this also.

Differences of settling in the liquid and in air are taken into consideration, since the flow in both cases would be viscous. Surface effects, viscosity effects would be ruled out.

Dimmick — Yes, but you couldn't have done this, for instance, with some spores because they just don't sink unless you pull a vacuum on them. Then if you do this you won't get their density in the air. We're talking about the density of a very dry spore which will be difficult to measure if put into a liquid.

McFarland — The Whitby settling technique is used more for defining the gross distributions in which particles vary from a few tenths of a micron up to something of the order of a hundred microns. In this case we must start with rather concentrated suspensions. I assume that we'd be talking of the order of a few spores. This might throw in a few complicating factors.

Lundgren — Dr. Goetz, I believe, spoke about the effect of air density on particle settling and stated that at 10 mb pressure, a 10 μ particle would fall like a rock. We've been doing some work in the 10 mb pressure range and find that 10 μ particles still can be transported easily. The Cunningham correction factor is still rather small, so particles don't settle out like rocks. They settle at a rate about three times faster than at atmospheric pressure.

[Editor's note: List of methods from blackboard:

1. Stirred settling chamber

2. "Galileo" method

3. Centrifugation

4. "Settling" in liquid. Pratt-Whitney centrifuge.]

Belmont — This question has nothing to do with the list of methods.

Where in the world might one find the best chance to locate biological material in the atmosphere? I suggest the tropics because at lower levels there are ideal conditions for biological life — warm temperatures, lots of humidity. Because of the convection in the tropics there's a good chance for upward transport. There's a far better chance in the tropics of locating biological materials in the stratosphere, or say the upper troposphere than there might be in mid or high latitudes. If one wants to start flying airplanes, as Dr. Junge suggested, this would certainly be a likely place to start. If particles are not to be found in the tropics, the chance of finding them elsewhere is much smaller, except in individual cases.

Junge — Of course, this is right; one should select the proper areas. In addition to this, one might consider even getting around airplane sampling by collecting at mountains above the trade wind inversion. There one can be relatively certain to have a representative sample of upper air at the earth surface. An excellent place is Mauna Loa Observatories in Hawaii. During the night there are subsiding air motions at the surface, so that contamination from the ground is unlikely.

Belmont — The only difficulty with such a location is, what is one likely to find? Surely, there will be descending motions maybe from very high levels, but this is in the middle of an ocean where the probability is relatively small that biological material will originate.

Junge — That's exactly what I meant because there one gets a representative sample from the upper troposphere, because there is no local influence from a continent. I would say this might be the type of representative material that one can expect to enter the stratosphere.

Belmont — It's a question of what we mean by "representative." We can find locations where the probability is high of finding biological material and we can find other locations where the probability is quite low. Which is representative, I don't know, except that I'm sure there is variability all over the earth's surface. If one wants hopefully to see if any biological material can exist at high levels he should go to the place where there are the best chances for it. If one does not find material there, his chance of finding it elsewhere is remote.

Junge — I do not agree quite with that. Three-quarters of the earth's surface is covered by ocean, so if one wants to know on the average what penetrates into the stratosphere it is best to select a place of this nature where most likely one will have an averaged population of particles which is more representative than one would collect over any other place. If this doesn't work here, perhaps one should select a few other places. This was just one offhand guess. The thing is that one can sample every night; one can get a wonderful seasonal variation.

Belmont — I agree with you. It's just that I think the problem is, can biological material exist at high levels in the atmosphere at all? If it can exist, then let's first look for it where there is good chance of finding the material.

I agree that the oceans cover most of the earth and that the conditions in Hawaii would be more representative of the globe than say, over Africa or India. But over Africa or India one might have a much higher chance of finding biological material. If it doesn't exist there, then one can likely conclude that the chance of finding it anywhere is remote.

Dimmick — What about the idea of spin drift in the ocean? Does anyone know how high water spouts go? Do they go into the stratosphere?

Belmont — I would doubt it very much. Hurricanes, thunderstorms, and other severe storms would have much more effect than water spouts. I think thunderstorms are the major agent for physically transporting things upward in a hurry, as they are so numerous.

Dimmick — Near the ocean one does find that the spin drift has put up salt crystals, as Dr. Goetz was mentioning. This possibly could be a large source of dried organisms. Also there is the potential of updrafts over the ocean. Actually this should be a rather large source, shouldn't it?

Junge — Salt crystals or matter is another subject. I am wondering about one thing. In our field of geophysics and meteorology we have a lot of international cooperation going on in the international scientific unions where there are committees to enhance global cooperation. Perhaps there is a similar scientific union for biologists that might be concerned with international programs called "coordination." Our problem really calls for some global coordination of scientific institutes and scientific people. This coordination might be one way to get things started a little better.

Benninghoff — The International Council of Scientific Unions (ICSU) for the past two years has been considering the possibility of an International Biological Programme [sic], a research effort that would extend over approximately seven years to accomplish synoptic inter-calibrated observations of biological productivity, biological resources, and human adaptability over the entire world.

In November 1963 ICSU adopted a basic proposal for the IBP and submitted it to academies of member nations of the International Union of Biological Sciences (IUBS). More than 30 nations have initiated study of the proposal. July 23 to 25, 1964, interested national academies and international bodies such as the Scientific Committee on Oceanographic Research (SCOR), the International Union for the Conservation of Nature (IUCN), and the Scientific Committee on Antarctic Research (SCAR) will send representatives to a meeting in Paris where the feasibility and organization of

an IBP will be deliberated. As planned the IBP would comprise sections for measurement of terrestrial, fresh water, and marine productivity, for processes of productivity, for human adaptability, and for use and management of biological resources. Human adaptability investigations will include studies of genetic and physiological bases for differential responses of various human groups to environmental stresses. Biological resources will take into account not only presently used raw materials but also potential source organisms and "genetic banks" for gene populations in danger of extinction.[1]

As far as I know there has been no attention given to aerobiology in planning for the IBP. This might be something to consider here, however. If there is some thought that within the structure of this proposed organization something could be done toward the coordination of international effort, it would be well for us to pass the information on to the United States committee (it is at present an *ad hoc* committee of the National Academy of Sciences under the chairmanship of Prof. Stanley A. Cain of the University of Michigan). This committee is making recommendations that presumably will result in the appointment of a duly constituted national committee in time for the July meeting in Paris.

Gregory — Mr. Chairman, this is a very practical suggestion; I should be glad to forward any such recommendations to the British committee.[2, 3]

Tsuchiya — I hope that the physical scientists will also be included in such a program; we have seen how fruitful interdisciplinary discussions can be.

Bruch — During the last comment about international cooperation, I was thinking about the International Geophysical Year in 1957 to 1958. The IGY participants were striving for international cooperation. Now as I understand it, this program was supervised through our National Academy of Science. Of course, the National Academy of Science has a Space Science Board, which could explore these cooperative scientific programs that we have discussed, in the hope of making them truly international in character.

When this Atmospheric Biology Conference was initially formulated I know that NASA's objectives were limited more or less to the problem of how to get a sterile spacecraft through our atmosphere and on its way to a planetary mission. Since that time NASA has broadened its approach to the study of microbial life in the upper atmosphere.

I have been pleased just as a scientist listening to the breadth of these discussions that we've had at this conference. I wish we could have explored more of the techniques and approaches used by the pollen researchers in their atmospheric studies.

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[1]Note added by Benninghoff, September 1964: "The First General Meeting on the IBP was held at UNESCO House as planned. About 30 nations sent delegates, some additional ones registered interest by correspondence, and all of the international scientific bodies that would be concerned were represented. The several sections worked out plans, time schedules, and committee memberships, which were then submitted to the parent committee in the form of recommendations. In plenary sessions a constitution was considered and adopted, and appointments were made to the Scientific Committee on the IBP (SCIBP) and to other offices. The IBP thus came into being and began its work."

[2]Note added by Gregory in proof: "Recommendations, together with a copy of Prof. W.S. Benninghoff's letter to Dr. Frank Campbell, were sent to the British National Committee for the International Biological Programme [sic] before the general I.B.P. meeting July 1964 in Paris. (P.H.G.)"

[3]Note added by Benninghoff, September, 1964: "In May 1964 Benninghoff wrote to the Executive Secretary of the National Academy Division of Biology and Agriculture inviting attention to the finding of this conference and to this discussion relating to the IBP. Gregory forwarded a copy of that letter under one of his own to the Royal Society Committee on the IBP. In the June 1964 report of the *ad hoc* committee of the National Academy of Sciences the following comment was made in discussion of the potential mission of Section E, Use and Management of Biological Resources: 'There is need for a study of atmospheric microflora by simultaneous observations at strategic locations around the world, taken at 'human level' and at designated levels above.' At The Paris meeting Section E deliberations resulted in a recommendation for studies of the atmosphere as a medium for international dissemination of plant and animal pathogens. In the Interim Report (Sept. 14, 1964) to the National Academy of Sciences by T.C. Byerly and S.A. Cain, co-chairmen of the NAS Delegation to the Paris meeting, the following item is reported among projects the Section E Working Group considered appropriate for the Section:

E. Ecology and Epidemiology of Plant Diseases

It is recommended that the SCIBP stress the need for and encourage investigations of the atmosphere as a medium of international dissemination of plant and animal pathogens. Examples would include studies of air currents and of animals such as migratory birds and bats as vectors of pathogens.

The ICSU Commission on Atmospheric Sciences is suggested as a cooperating agency.

Gregory and Benninghoff are communicating with other investigators of air spora and inviting their attention to these developments."

Another strong aspect of the conference has been the methodology and instrumentation which have been discussed quite broadly.

Any time any of you want more information about NASA's activities in space, or want to find out what NASA can do to stimulate research in space, feel free to contact me in Washington, or to contact Mr. MacLeod. The mission of our office, the Office of Space Sciences and Applications, is to learn more about space and what is occurring there. We want to encourage anything that supports that mission.

Literature Citation

A. STORM VAN LEEUWEN, W., Z. BIEN, & H. VAREKAMP. 1925. Zeitschr. f. Immun.-forsch. 43: 490.

General Nomenclature

Alt	Altitude
A	Angstrom units
acfm	Ambient cubic feet per minute
AGI	All glass impinger
C	Centigrade (degree symbol is omitted); Celsius
C	Cunningham correction factor
cfm	Cubic feet per minute
cfs	Cubic feet per second
cpm	Counts per minute
CP	Constant pressure
cps	Centimeters per second
CWAS	Cryogenic whole air sampler
dc	Direct current
DFS	Direct flow sampler
DPA	Dipicolinic acid
dpm	Disintegrations per minute
esu	Electrostatic units
F	Fahrenheit (degree symbol is omitted)
fpm	Feet per minute
fps	Feet per second
g	Gravity
IPC	Institute of Paper Chemistry
k	Boltzmann's constant
K	Kelvin (degree symbol is omitted)
kv	Kilovolts
Lat	Latitude
λ	Length of mean free path
Long	Longitude
m	Mass
m	Meters
mb	Millibar
meq	Milliequivalent
Mev	Million electron volts
MF	Membrane filter
Mscf	Thousand cubic feet
ppm	Parts per million
psi	Pounds per square inch
psig	Pounds per square inch gauge
RH	Relative humidity
rpm	Revolutions per minute
rps	Revolutions per second
scf	Standard cubic feet
scfm	Standard cubic feet per minute
STP	Standard temperature & pressure
t	Time
T	Temperature
UV	Ultraviolet
V	Velocity